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(54) Title: REGULATION OF HUMAN ADAM-TS-LIKE ZINC METALLOPROTEASE

(57) Abstract: Reagents that regulate human ADAM-TS-like zinc metalloprotease and reagents which bind to human ADAM-TS-like zinc metalloprotease gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, COPD, CNS disorders, and cardiovascular disorders.

TECHNICAL FIELD OF THE INVENTION

The invention relates to the regulation of human ADAM-TS-like zinc metallo-protease.

BACKGROUND OF THE INVENTION

ADAMs are a family of novel membrane-spanning multi-domain proteins containing a zinc metalloproteinase domain and a disintegrin domain which may serve as an integrin ligand. Hurskainen *et al.*, *J. Biol. Chem. 274*, 25555-63, 1999; Kuo *et al.*, *J. Biol. Chem. 274*, 18821-26, 1999; Kuno *et al.*, *J. Biol. Chem. 272*, 556-62, 1997; Stone *et al.*, *J. Protein Chem. 18*, 447-65, 1999; Millichip *et al.*, *Biochem. Biophys. Res. Commun. 245*, 594-98, 1998. Some ADAMs are involved in the shedding and activation of cytokines and growth factors such as TNF-β. Inflammatory processes in the liver which eventually result in liver fibrosis are frequently induced by the activation of TNF-β. Other ADAMs are associated with the extracellular matrix and play other roles in inflammatory processes. For example, type IV collagenase activity is associated with some ADAMS. It is known that migration and activation of fibrogenic hepatic stellate cells is functionally linked to type IV collagenase activity. It is therefore reasonable to assume that more novel genes of this family will be detected, the products of which might be functionally involved with the induction or propagation of liver fibrosis. Therefore, novel ADAMs will be good targets for therapeutic intervention in liver fibrosis. ADAMs also may be useful for treating cardiovascular disease. There is, therefore, a continuing need in the art to identify members of this protein family which can be regulated to provide therapeutic effects.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating a human ADAM-TS-like zinc metalloprotease. This and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a ADAM-TS-like zinc metalloprotease polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a ADAM-TS-like zinc metalloprotease polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

Binding between the test compound and the ADAM-TS-like zinc metalloprotease polypeptide is detected. A test compound which binds to the ADAM-TS-like zinc metalloprotease polypeptide is thereby identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the activity of the ADAM-TS-like zinc metalloprotease.

Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a ADAM-TS-like zinc metalloprotease polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the ADAM-TS-like zinc metalloprotease through interacting with the ADAM-TS-like zinc metalloprotease mRNA.

Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a ADAM-TS-like zinc metalloprotease polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

A ADAM-TS-like zinc metalloprotease activity of the polypeptide is detected. A test compound which increases ADAM-TS-like zinc metalloprotease activity of the polypeptide relative to ADAM-TS-like zinc metalloprotease activity in the absence of the test compound is thereby identified as a potential agent for increasing

extracellular matrix degradation. A test compound which decreases ADAM-TS-like zinc metalloprotease activity of the polypeptide relative to ADAM-TS-like zinc metalloprotease activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a ADAM-TS-like zinc metalloprotease product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

Binding of the test compound to the ADAM-TS-like zinc metalloprotease product is detected. A test compound which binds to the ADAM-TS-like zinc metalloprotease product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a ADAM-TS-like zinc metalloprotease polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

ADAM-TS-like zinc metalloprotease activity in the cell is thereby decreased.

The invention thus provides a human ADAM-TS-like zinc metalloprotease that can be used to identify test compounds that may act, for example, as activators or inhibitors at the enzyme's active site. Human ADAM-TS-like zinc metalloprotease and fragments thereof also are useful in raising specific antibodies that can block the enzyme and effectively reduce its activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the DNA-sequence encoding a ADAM-TS-like zinc metallo-protease polypeptide (SEQ ID NO:1).

Fig. 2 shows the amino acid sequence deduced from the DNA-sequence of Fig.1 (SEQ ID NO:2).

Fig. 3 shows the amino acid sequence of the protein identified by Swissnew Accession No. P97857|ATS1_MOUSE ADAM-TS 1 PRECURSOR (SEQ ID NO:3).

Fig. 4 shows the DNA-sequence encoding a ADAM-TS-like zinc metallo-protease polypeptide (SEQ ID NO:4).

Fig. 5 shows the BLASTP - alignment of 367_protein (SEQ ID NO:2) against swissnew|P97857|ATS1_MOUSE ADAM-TS 1 PRECURSOR (EC 3.4.24.-) (SEQ ID NO:3).

Fig. 6 shows the BLASTP - alignment of 367_ (SEQ ID NO:2) against trembl|AF140675|AF140675_1 gene: "ADAMTS7" (SEQ ID NO:4).

Fig. 7 shows the BLASTP - alignment of 367_protein (SEQ ID NO:2) against pdb|1ATL|1ATL-A.

Fig. 8 shows the HMMPFAM - alignment of 367_protein (SEQ ID NO:2) against pfam|hmm|Reprolysin.

Fig. 9 shows the HMMPFAM - alignment of 367_protein (SEQ ID NO:2) against pfam|hmm|tsp_1.

Fig. 10 shows the relative expression of ADAM TS-like mRNA in various human tissues.

Fig. 11 shows the relative expression of ADAM TS-like gene in various human respiratory tissues and cells (HBEC=cultured human bronchial epithelial cells; H441=Clara-like cells; SMC=cultured airway smooth muscle cells; SAE= cultured small airway epithelial cells; AII=primary cultured alveolar type II cells; PMN=polymorphonuclear leukocytes; Mono=monocytes; Cult. Mono=cultured monocytes (macrophage-like).

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated polynucleotide being selected from the group consisting of:

a) a polynucleotide encoding a ADAM-TS-like zinc metalloprotease polypeptide comprising an amino acid sequence selected from the group consisting of:
amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO: 2; and
the amino acid sequence shown in SEQ ID NO: 2.

b) a polynucleotide comprising the sequence of SEQ ID NO: 1;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b) and encodes a ADAM-TS-like zinc metalloprotease polypeptide;

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes a ADAM-TS-like zinc metalloprotease polypeptide; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and encodes a ADAM-TS-like zinc metalloprotease polypeptide.

to swissnew|P97857|ATS1_MOUSE ADAM-TS 1 PRECURSOR (EC 3.4.24.-) (SEQ ID NO:3) (Fig. 5). Human ADAM-TS-like zinc metalloprotease also is 44% identical over 565 amino acids to trembl|AF140675|AF140675_1 gene: "ADAMTS7" (SEQ ID NO:4) (Fig. 6). The functional assignment of the protein is based on its similarity to the zinc metalloproteinase region and the thrombospondin domain of ADAM-TS 1 precursor (EC 3.4.24.-), a disintegrain and metalloproteinase with thrombospondin motifs (ADAM-TS-1). This protein also shares good similarity with other members of the ADAM-TS family. Three-dimensional homology and PFAM searches also support the identification of this protein as an ADAM-TS-like zinc metalloprotease.

Human ADAM-TS-like zinc metalloprotease of the invention is expected to be useful for the same purposes as previously identified ADAM-TS-like zinc metalloprotease enzymes. Human ADAM-TS-like zinc metalloprotease is believed to be useful in therapeutic methods to treat disorders such as COPD, cardiovascular disorders, and CNS disorders. Human ADAM-TS-like zinc metalloprotease also can be used to screen for human ADAM-TS-like zinc metalloprotease activators and inhibitors.

Polypeptides

Human ADAM-TS-like zinc metalloprotease polypeptides according to the invention comprise at least 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 350, 400, 450, 500, 550, or 556 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:2 or a biologically active variant thereof, as defined below. A ADAM-TS-like zinc metalloprotease polypeptide of the invention therefore can be a portion of a ADAM-TS-like zinc metalloprotease protein, a full-length ADAM-TS-like zinc metalloprotease protein, or a fusion protein comprising all or a portion of a ADAM-TS-like zinc metalloprotease protein.

Biologically Active Variants

Human ADAM-TS-like zinc metalloprotease polypeptide variants that are biologically active, *e.g.*, retain a zinc metalloprotease activity, also are ADAM-TS-like zinc metalloprotease polypeptides. Preferably, naturally or non-naturally occurring ADAM-TS-like zinc metalloprotease polypeptide variants have amino acid sequences which are at least about 45, 50, 55, 60, 65, or 70, preferably about 75, 80, 85, 90, 96, 96, 98, or 99% identical to the amino acid sequence shown in SEQ ID NO:2 or a fragment thereof. Percent identity between a putative ADAM-TS-like zinc metalloprotease polypeptide variant and an amino acid sequence of SEQ ID NO:2 is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48:603 (1986), and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM62" scoring matrix of Henikoff and Henikoff (ibid.).Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA"similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative variant. The FASTA algorithm is described y Pearson and Lipman, Proc.

Nat'l Acad. Sci. USA 85:2444(1988), and by Pearson, Meth. Enzymol. 183:63 (1990).Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (e.g. SEQ ID NO: 2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to for man approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch- Sellers algorithm (Needleman and Wunsch, J. Mol. Biol.48:444 (1970); Sellers, SIAM J. Appl. Math. 26:787 (1974)), which allows for amino acid insertions and deletions. Preferred parameters for FASTA analysis are: ktup=1, gapopeningpenalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, Meth. Enzymol. 183:63 (1990).FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as default.

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a ADAM-TS-like zinc metalloprotease polypeptide can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active ADAM-TS-like zinc metalloprotease polypeptide can readily be determined by assaying for enzyme activity. See See, *e.g.*, Black & White, *Curr. Opin. Cell Biol. 10*, 654-59, 1998.

Fusion Proteins

Fusion proteins are useful for generating antibodies against ADAM-TS-like zinc metalloprotease polypeptide amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins that interact with portions of a ADAM-TS-like zinc metalloprotease polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

A ADAM-TS-like zinc metalloprotease polypeptide fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment comprises at 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 350, 400, 450, 500, 550, or 556 contiguous amino acids of SEQ ID NO:2 or of a biologically active variant, such as those described above. The first polypeptide segment also can comprise full-length ADAM-TS-like zinc metalloprotease protein.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include β-galactosidase, β-

the ADAM-TS-like zinc metalloprotease polypeptide-encoding sequence and the heterologous protein sequence, so that the ADAM-TS-like zinc metalloprotease polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from SEQ ID NO:1 in proper reading frame with nucleotides encoding the second polypeptide segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

Species homologs of human ADAM-TS-like zinc metalloprotease polypeptide can be obtained using ADAM-TS-like zinc metalloprotease polypeptide polynucleotides (described below) to make suitable probes or primers for screening cDNA expression

libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of ADAM-TS-like zinc metalloprotease polypeptide, and expressing the cDNAs as is known in the art.

Polynucleotides

A ADAM-TS-like zinc metalloprotease polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a ADAM-TS-like zinc metalloprotease polypeptide. A coding sequence for human ADAM-TS-like zinc metalloprotease is shown in SEQ ID NO:1.

Degenerate nucleotide sequences encoding human ADAM-TS-like zinc metalloprotease polypeptides, as well as homologous nucleotide sequences which are at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, 98, or 99% identical to the nucleotide sequence shown in SEQ ID NO:1 or its complement also are ADAM-TS-like zinc metalloprotease polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of ADAM-TS-like zinc metalloprotease polynucleotides that encode biologically active ADAM-TS-like zinc metalloprotease polypeptides also are ADAM-TS-like zinc metalloprotease polynucleotides. Polynucleotide fragments comprising at least 8, 9, 10, 11, 12, 15, 20, or 25 contiguous nucleotides of SEQ ID NO:1 or its complement also are ADAM-TS-like zinc metalloprotease polynucleotides. These fragments can be used, for example, as hybridization probes or as antisense oligonucleotides.

Identification of Polynucleotide Variants and Homologs

Variants and homologs of the ADAM-TS-like zinc metalloprotease polynucleotides described above also are ADAM-TS-like zinc metalloprotease polynucleotides.

Typically, homologous ADAM-TS-like zinc metalloprotease polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known ADAM-TS-like zinc metalloprotease polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50 °C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the ADAM-TS-like zinc metalloprotease polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of ADAM-TS-like zinc metalloprotease polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5 °C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol. 81*, 123 (1973). Variants of human ADAM-TS-like zinc metalloprotease polynucleotides or ADAM-TS-like zinc metalloprotease polynucleotides of other species can therefore be identified by hybridizing a putative homologous ADAM-TS-like zinc metalloprotease polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO:1 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to ADAM-TS-like zinc metalloprotease polynucleotides or their complements following stringent hybridization and/or wash conditions also are ADAM-TS-like zinc metalloprotease polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for

example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20 °C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a ADAM-TS-like zinc metalloprotease polynucleotide having a nucleotide sequence shown in SEQ ID NO:1 or the complement thereof and a polynucleotide sequence which is at least about 50, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A. 48*, 1390 (1962):

$T_m = 81.5\ °C - 16.6(\log_{10}[Na^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l$,
where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65 °C, or 50% formamide, 4X SSC at 42 °C, or 0.5X SSC, 0.1% SDS at 65 °C. Highly stringent wash conditions include, for example, 0.2X SSC at 65 °C.

Preparation of Polynucleotides

A ADAM-TS-like zinc metalloprotease polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated ADAM-TS-like zinc metalloprotease polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments, which comprise ADAM-TS-like zinc

like zinc metalloprotease polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a ADAM-TS-like zinc metalloprotease polypeptide having, for example, an amino acid sequence shown in SEQ ID NO:2 or a biologically active variant thereof.

Extending Polynucleotides

The partial sequence disclosed herein can be used to identify the corresponding full length gene from which it was derived. The partial sequence can be nick-translated or end-labeled with ^{32}P using polynucleotide kinase using labeling methods known to those with skill in the art (BASIC METHODS IN MOLECULAR BIOLOGY, Davis *et al.*, eds., Elsevier Press, N.Y., 1986). A lambda library prepared from human tissue can be directly screened with the labeled sequences of interest or the library can be converted en masse to pBluescript (Stratagene Cloning Systems, La Jolla, Calif. 92037) to facilitate bacterial colony screening (see Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory Press (1989, pg. 1.20).

Both methods are well known in the art. Briefly, filters with bacterial colonies containing the library in pBluescript or bacterial lawns containing lambda plaques are denatured, and the DNA is fixed to the filters. The filters are hybridized with the labeled probe using hybridization conditions described by Davis *et al.*, 1986. The partial sequences, cloned into lambda or pBluescript, can be used as positive controls to assess background binding and to adjust the hybridization and washing stringencies necessary for accurate clone identification. The resulting autoradiograms are compared to duplicate plates of colonies or plaques; each exposed spot corresponds to a positive colony or plaque. The colonies or plaques are selected, expanded and the DNA is isolated from the colonies for further analysis and sequencing.

Positive cDNA clones are analyzed to determine the amount of additional sequence they contain using PCR with one primer from the partial sequence and the other primer from the vector. Clones with a larger vector-insert PCR product than the original partial sequence are analyzed by restriction digestion and DNA sequencing to determine whether they contain an insert of the same size or similar as the mRNA size determined from Northern blot Analysis.

Once one or more overlapping cDNA clones are identified, the complete sequence of the clones can be determined , for example after exonuclease III digestion (McCombie *et al.*, *Methods 3*, 33-40, 1991). A series of deletion clones are generated, each of which is sequenced. The resulting overlapping sequences are assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a highly accurate final sequence.

Various PCR-based methods can be used to extend the nucleic acid sequences disclosed herein to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic. 2*,

Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72 °C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic. 1*, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 3055-3060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in

that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) that are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (*e.g.* GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA that might be present in limited amounts in a particular sample.

Obtaining Polypeptides

Human ADAM-TS-like zinc metalloprotease polypeptides can be obtained, for example, by purification from human cells, by expression of ADAM-TS-like zinc metalloprotease polynucleotides, or by direct chemical synthesis.

Protein Purification

Human ADAM-TS-like zinc metalloprotease polypeptides can be purified from any cell that expresses the polypeptide, including host cells that have been transfected with ADAM-TS-like zinc metalloprotease expression constructs. A purified ADAM-TS-like zinc metalloprotease polypeptide is separated from other compounds that normally associate with the ADAM-TS-like zinc metalloprotease polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in

can be inserted into an expression vector that contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods that are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding ADAM-TS-like zinc metalloprotease polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding a ADAM-TS-like zinc metalloprotease polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO, and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a ADAM-TS-like zinc metalloprotease polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the ADAM-TS-like zinc metalloprotease polypeptide. For example, when a large quantity of a ADAM-TS-like zinc metalloprotease polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the ADAM-TS-like zinc metalloprotease polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem. 264*, 5503-5509, 1989) or pGEX vectors (Promega,

For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol. 153*, 516-544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding ADAM-TS-like zinc metalloprotease polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J. 6*, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J. 3*, 1671-1680, 1984; Broglie *et al.*, *Science 224*, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ. 17*, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (*e.g.*, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express a ADAM-TS-like zinc metalloprotease polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera*

frugiperda cells or in *Trichoplusia* larvae. Sequences encoding ADAM-TS-like zinc metalloprotease polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of ADAM-TS-like zinc metalloprotease polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda cells* or *Trichoplusia* larvae in which ADAM-TS-like zinc metalloprotease polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci. 91*, 3224-3227, 1994).

Mammalian Expression Systems

A number of viral-based expression systems can be used to express ADAM-TS-like zinc metalloprotease polypeptides in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding ADAM-TS-like zinc metalloprotease polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus that is capable of expressing a ADAM-TS-like zinc metalloprotease polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci. 81*, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (*e.g.*, liposomes, polycationic amino polymers, or vesicles).

Specific initiation signals also can be used to achieve more efficient translation of sequences encoding ADAM-TS-like zinc metalloprotease polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a ADAM-TS-like zinc metalloprotease polypeptide, its initiation codon,

and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ. 20*, 125-162, 1994).

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed ADAM-TS-like zinc metalloprotease polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells that have specific cellular machinery and characteristic mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express ADAM-TS-like zinc metalloprotease polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they

are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced ADAM-TS-like zinc metalloprotease sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

Any number of selection systems can be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*, 817-23, 1980) genes which can be employed in *tk*$^-$ or *aprt*$^-$ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci. 77*, 3567-70, 1980), *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol. 150*, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. 85*, 8047-51, 1988). Visible markers such as anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol. 55*, 121-131, 1995).

Detecting Expression

Although the presence of marker gene expression suggests that the ADAM-TS-like zinc metalloprotease polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a ADAM-TS-like zinc metalloprotease polypeptide is inserted within a marker gene sequence, transformed

cells containing sequences that encode a ADAM-TS-like zinc metalloprotease polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a ADAM-TS-like zinc metalloprotease polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the ADAM-TS-like zinc metalloprotease polynucleotide.

Alternatively, host cells which contain a ADAM-TS-like zinc metalloprotease polynucleotide and which express a ADAM-TS-like zinc metalloprotease polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques that include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding a ADAM-TS-like zinc metalloprotease polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding a ADAM-TS-like zinc metalloprotease polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a ADAM-TS-like zinc metalloprotease polypeptide to detect transformants that contain a ADAM-TS-like zinc metalloprotease polynucleotide.

A variety of protocols for detecting and measuring the expression of a ADAM-TS-like zinc metalloprotease polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a ADAM-TS-like zinc metalloprotease polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et*

al., SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med. 158*, 1211-1216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding ADAM-TS-like zinc metalloprotease polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a ADAM-TS-like zinc metalloprotease polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, co-factors, inhibitors, magnetic particles, and the like.

Expression and Purification of Polypeptides

Host cells transformed with nucleotide sequences encoding a ADAM-TS-like zinc metalloprotease polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode ADAM-TS-like zinc metalloprotease polypeptides can be designed to contain signal sequences which direct secretion of soluble ADAM-TS-like zinc metalloprotease polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound ADAM-TS-like zinc metalloprotease polypeptide.

As discussed above, other constructions can be used to join a sequence encoding a ADAM-TS-like zinc metalloprotease polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the ADAM-TS-like zinc metalloprotease polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a ADAM-TS-like zinc metalloprotease polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif. 3*, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the ADAM-TS-like zinc metalloprotease polypeptide from the fusion protein. Vectors that contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol. 12*, 441-453, 1993.

Chemical Synthesis

Sequences encoding a ADAM-TS-like zinc metalloprotease polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al. Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a ADAM-TS-like zinc metalloprotease polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc. 85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be performed using manual

techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of ADAM-TS-like zinc metalloprotease polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic ADAM-TS-like zinc metalloprotease polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the ADAM-TS-like zinc metalloprotease polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce ADAM-TS-like zinc metalloprotease polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life that is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter ADAM-TS-like zinc metalloprotease polypeptide-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of

gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of a ADAM-TS-like zinc metalloprotease polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv, which are capable of binding an epitope of a ADAM-TS-like zinc metalloprotease polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

An antibody which specifically binds to an epitope of a ADAM-TS-like zinc metalloprotease polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody that specifically binds to the immunogen.

Typically, an antibody which specifically binds to a ADAM-TS-like zinc metalloprotease polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to ADAM-TS-like zinc metalloprotease polypeptides do not detect other proteins in immunochemical

assays and can immunoprecipitate a ADAM-TS-like zinc metalloprotease polypeptide from solution.

Human ADAM-TS-like zinc metalloprotease polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a ADAM-TS-like zinc metalloprotease polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

Monoclonal antibodies that specifically bind to a ADAM-TS-like zinc metalloprotease polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature 256*, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods 81*, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci. 80*, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol. 62*, 109-120, 1984).

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*, 6851-6855, 1984; Neuberger *et al.*, *Nature 312*, 604-608, 1984; Takeda *et al.*, *Nature 314*, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be

humanized antibodies can be produced using recombinant methods, as described in GB2188638B. Antibodies that specifically bind to a ADAM-TS-like zinc metallo-protease polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies that specifically bind to ADAM-TS-like zinc metalloprotease polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci. 88*, 11120-23, 1991).

Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev. 5*, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol. 15*, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem. 269*, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*,

1995, *Int. J. Cancer 61*, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth. 165*, 81-91).

Antibodies which specifically bind to ADAM-TS-like zinc metalloprotease polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci. 86*, 3833-3837, 1989; Winter *et al.*, *Nature 349*, 293-299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a ADAM-TS-like zinc metalloprotease polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences that are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a

mated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. *See* Brown, *Meth. Mol. Biol. 20*, 1-8, 1994; Sonveaux, *Meth. Mol. Biol. 26*, 1-72, 1994; Uhlmann *et al.*, *Chem. Rev. 90*, 543-583, 1990.

Modifications of ADAM-TS-like zinc metalloprotease gene expression can be obtained by designing antisense oligonucleotides that will form duplexes to the control, 5', or regulatory regions of the ADAM-TS-like zinc metalloprotease gene. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a ADAM-TS-like zinc metalloprotease polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a ADAM-TS-like zinc metalloprotease polynucleotide, each

separated by a stretch of contiguous nucleotides which are not complementary to adjacent ADAM-TS-like zinc metalloprotease nucleotides, can provide sufficient targeting specificity for ADAM-TS-like zinc metalloprotease mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular ADAM-TS-like zinc metalloprotease polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a ADAM-TS-like zinc metalloprotease polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g.*, Agrawal *et al.*, *Trends Biotechnol. 10*, 152-158, 1992; Uhlmann *et al.*, *Chem. Rev. 90*, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett. 215*, 3539-3542, 1987.

Ribozymes

Ribozymes are RNA molecules with catalytic activity. *See, e.g.*, Cech, *Science 236*, 1532-1539; 1987; Cech, *Ann. Rev. Biochem. 59*, 543-568; 1990, Cech, *Curr. Opin. Struct. Biol. 2*, 605-609; 1992, Couture & Stinchcomb, *Trends Genet. 12*, 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g.*, Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme

molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a ADAM-TS-like zinc metalloprotease polynucleotide can be used to generate ribozymes that will specifically bind to mRNA transcribed from the ADAM-TS-like zinc metalloprotease polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (*see* Haseloff *et al. Nature 334*, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within a ADAM-TS-like zinc metalloprotease RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate ADAM-TS-like zinc metalloprotease RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease ADAM-TS-like zinc metalloprotease expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors that induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Differentially Expressed Genes

Described herein are methods for the identification of genes whose products interact with human ADAM-TS-like zinc metalloprotease. Such genes may represent genes that are differentially expressed in disorders including, but not limited to, COPD, cardiovascular disorders, and CNS disorders. Further, such genes may represent genes that are differentially regulated in response to manipulations relevant to the progression or treatment of such diseases. Additionally, such genes may have a temporally modulated expression, increased or decreased at different stages of tissue or organism development. A differentially expressed gene may also have its expression modulated under control versus experimental conditions. In addition, the human ADAM-TS-like zinc metalloprotease gene or gene product may itself be tested for differential expression.

The degree to which expression differs in a normal versus a diseased state need only be large enough to be visualized via standard characterization techniques such as differential display techniques. Other such standard characterization techniques by which expression differences may be visualized include but are not limited to, quantitative RT (reverse transcriptase), PCR, and Northern analysis.

Identification of Differentially Expressed Genes

To identify differentially expressed genes total RNA or, preferably, mRNA is isolated from tissues of interest. For example, RNA samples are obtained from tissues of experimental subjects and from corresponding tissues of control subjects. Any RNA isolation technique that does not select against the isolation of mRNA may be utilized for the purification of such RNA samples. See, for example, Ausubel *et al.*, ed., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc. New York, 1987-1993. Large numbers of tissue samples may readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, U.S. Patent 4,843,155.

Transcripts within the collected RNA samples that represent RNA produced by differentially expressed genes are identified by methods well known to those of skill in the art. They include, for example, differential screening (Tedder *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 85*, 208-12, 1988), subtractive hybridization (Hedrick *et al.*, *Nature 308*, 149-53; Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 88*, 2825, 1984), and, preferably, differential display (Liang & Pardee, *Science 257*, 967-71, 1992; U.S. Patent 5,262,311).

The differential expression information may itself suggest relevant methods for the treatment of disorders involving the human ADAM-TS-like zinc metalloprotease. For example, treatment may include a modulation of expression of the differentially expressed genes and/or the gene encoding the human ADAM-TS-like zinc metalloprotease. The differential expression information may indicate whether the

expression or activity of the differentially expressed gene or gene product or the human ADAM-TS-like zinc metalloprotease gene or gene product are up-regulated or down-regulated.

Screening Methods

The invention provides assays for screening test compounds that bind to or modulate the activity of a ADAM-TS-like zinc metalloprotease polypeptide or a ADAM-TS-like zinc metalloprotease polynucleotide. A test compound preferably binds to a ADAM-TS-like zinc metalloprotease polypeptide or polynucleotide. More preferably, a test compound decreases or increases ADAM-TS-like zinc metalloprotease activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. *See* Lam, *Anticancer Drug Des. 12*, 145, 1997.

(Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* *89*, 1865-1869, 1992), or phage (Scott & Smith, *Science 249*, 386-390, 1990; Devlin, *Science 249*, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci. 97*, 6378-6382, 1990; Felici, *J. Mol. Biol. 222*, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

High Throughput Screening

Test compounds can be screened for the ability to bind to ADAM-TS-like zinc metalloprotease polypeptides or polynucleotides or to affect ADAM-TS-like zinc metalloprotease activity or ADAM-TS-like zinc metalloprotease gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 µl. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 19*, 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds

are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity 2*, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

Binding Assays

For binding assays, the test compound is preferably a small molecule that binds to and occupies, for example, the active site of the ADAM-TS-like zinc metalloprotease polypeptide, such that normal biological activity is prevented. Examples of such

small molecules include, but are not limited to, small peptides or peptide-like molecules.

In binding assays, either the test compound or the ADAM-TS-like zinc metalloprotease polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound that is bound to the ADAM-TS-like zinc metalloprotease polypeptide can then be accomplished, for example, by direct counting of radioemmission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

Alternatively, binding of a test compound to a ADAM-TS-like zinc metalloprotease polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with a ADAM-TS-like zinc metalloprotease polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and a ADAM-TS-like zinc metalloprotease polypeptide (McConnell *et al.*, *Science 257*, 1906-1912, 1992).

Determining the ability of a test compound to bind to a ADAM-TS-like zinc metalloprotease polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem. 63*, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol. 5*, 699-705, 1995). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, a ADAM-TS-like zinc metalloprotease polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell 72*, 223-232, 1993; Madura *et al.*, *J. Biol. Chem. 268*, 12046-12054, 1993; Bartel *et al.*, *BioTechniques 14*, 920-924, 1993; Iwabuchi *et al.*, *Oncogene 8*, 1693-1696, 1993; and Brent W094/10300), to identify other proteins which bind to or interact with the ADAM-TS-like zinc metalloprotease polypeptide and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding a ADAM-TS-like zinc metalloprotease polypeptide can be fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein that interacts with the ADAM-TS-like zinc metalloprotease polypeptide.

It may be desirable to immobilize either the ADAM-TS-like zinc metalloprotease polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the ADAM-TS-like zinc metalloprotease polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or

plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the enzyme polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to a ADAM-TS-like zinc metalloprotease polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, the ADAM-TS-like zinc metalloprotease polypeptide is a fusion protein comprising a domain that allows the ADAM-TS-like zinc metalloprotease polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed ADAM-TS-like zinc metalloprotease polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a ADAM-TS-like zinc metalloprotease polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated ADAM-TS-like zinc metalloprotease polypeptides (or polynucleotides) or test compounds can be prepared from biotin-NHS(N-hydroxysuccinimide) using techniques

well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a ADAM-TS-like zinc metalloprotease polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the active site of the ADAM-TS-like zinc metalloprotease polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the ADAM-TS-like zinc metalloprotease polypeptide or test compound, enzyme-linked assays which rely on detecting an activity of the ADAM-TS-like zinc metalloprotease polypeptide, and SDS gel electrophoresis under non-reducing conditions.

Screening for test compounds which bind to a ADAM-TS-like zinc metalloprotease polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a ADAM-TS-like zinc metalloprotease polypeptide or polynucleotide can be used in a cell-based assay system. A ADAM-TS-like zinc metalloprotease polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the test compound to a ADAM-TS-like zinc metalloprotease polypeptide or polynucleotide is determined as described above.

Enzyme Assays

Test compounds can be tested for the ability to increase or decrease the enzyme activity of a human ADAM-TS-like zinc metalloprotease polypeptide. Enzyme activity can be measured, for example, as described in See, *e.g.*, Black & White, *Curr. Opin. Cell Biol. 10*, 654-59, 1998.

for decreasing ADAM-TS-like zinc metalloprotease activity. A test compound which increases an enzyme activity of a human ADAM-TS-like zinc metalloprotease polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for increasing human ADAM-TS-like zinc metalloprotease activity.

Gene Expression

In another embodiment, test compounds that increase or decrease ADAM-TS-like zinc metalloprotease gene expression are identified. A ADAM-TS-like zinc metalloprotease polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the ADAM-TS-like zinc metalloprotease polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of ADAM-TS-like zinc metalloprotease mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence

of polypeptide products of a ADAM-TS-like zinc metalloprotease polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a ADAM-TS-like zinc metalloprotease polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell that expresses a ADAM-TS-like zinc metalloprotease polynucleotide can be used in a cell-based assay system. The ADAM-TS-like zinc metalloprotease polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, such as CHO or human embryonic kidney 293 cells, can be used.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions that can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, a ADAM-TS-like zinc metalloprotease polypeptide, ADAM-TS-like zinc metalloprotease polynucleotide, ribozymes or antisense oligonucleotides, antibodies which specifically bind to a ADAM-TS-like zinc metalloprotease polypeptide, or mimetics, activators, or inhibitors of a ADAM-TS-like zinc metalloprotease polypeptide activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries

that facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxy-propylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as

glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Human ADAM-TS-like zinc metalloprotease can be regulated to treat COPD, cardiovascular disorders, and CNS disorders.

The relative expression of ADAM TS-like gene across various human tissues is shown in Fig. 10. Expression of the gene was detected in many of the tissues tested, including lung, and was especially abundant in kidney and uterus. Expression of ADAM TS-like gene in lung was of particular interest and this was investigated further by analysis of the expression of the gene in some of the constituent cell types of the lung. In these samples, expression was detected in only the Clara-like cell line H441 and alveolar type II cells (Fig. 11). The gene was not expressed in any of the inflammatory cell types tested.

The expression of ADAM TS-like protein is consistent with a function in tissue remodeling. Dysfunction or dysregulation of the protease plays a potentially significant role in the destruction of the lung matrix in diseases such as COPD. ADAM TS-like protein, therefore, represents a therapeutic target for COPD.

Chronic obstructive pulmonary (or airways) disease (COPD) is a condition defined physiologically as airflow obstruction that generally results from a mixture of emphysema and peripheral airway obstruction due to chronic bronchitis (Senior & Shapiro, *Pulmonary Diseases and Disorders*, 3d ed., New York, McGraw-Hill, 1998,

pp. 659-681, 1998; Barnes, *Chest 117*, 10S-14S, 2000). Emphysema is characterized by destruction of alveolar walls leading to abnormal enlargement of the air spaces of the lung. Chronic bronchitis is defined clinically as the presence of chronic productive cough for three months in each of two successive years. In COPD, airflow obstruction is usually progressive and is only partially reversible. By far the most important risk factor for development of COPD is cigarette smoking, although the disease does occur in non-smokers.

Chronic inflammation of the airways is a key pathological feature of COPD (Senior & Shapiro, 1998). The inflammatory cell population comprises increased numbers of macrophages, neutrophils, and $CD8^+$ lymphocytes. Inhaled irritants, such as cigarette smoke, activate macrophages which are resident in the respiratory tract, as well as epithelial cells leading to release of chemokines (*e.g.*, interleukin-8) and other chemotactic factors. These chemotactic factors act to increase the neutrophil/monocyte trafficking from the blood into the lung tissue and airways. Neutrophils and monocytes recruited into the airways can release a variety of potentially damaging mediators such as proteolytic enzymes and reactive oxygen species. Matrix degradation and emphysema, along with airway wall thickening, surfactant dysfunction, and mucus hypersecretion, all are potential sequelae of this inflammatory response that lead to impaired airflow and gas exchange.

COPD is characterized by damage to the lung extracellular matrix and emphysema can be viewed as the pathologic process that affects the lung parenchyma. This process eventually leads to the destruction of the airway walls resulting in permanent airspace enlargement (Senior and Shapiro, in PULMONARY DISEASES AND DISORDERS, 3rd ed., New York, McGraw-Hill, 1998, pp. 659 – 681, 1998). The observation that inherited deficiency of a1-antitrypsin (a1-AT), the primary inhibitor of neutrophil elastase, predisposes individuals to early onset emphysema, and that intrapulmonary instillation of elastolytic enzymes in experimental animals causes emphysema, led to the elastase:antielastase hypothesis for the pathogenesis of emphysema (Eriksson, *Acta Med. Scand. 177(Suppl.)*, 432, 1965, Gross, *J. Occup.*

Med. 6, 481-84, 1964). This in turn led to the concept that destruction of elastin in the lung parenchyma is the basis of the development of emphysema.

A broad range of immune and inflammatory cells including neutrophils, macrophages, T lymphocytes and eosinophils contain proteolytic enzymes that could contribute to the destruction of lung extracellular matrix (Shapiro, 1999). In addition, a number of different classes of proteases have been identified that have the potential to contribute to lung matrix destruction. These include serine proteases, matrix metalloproteinases and cysteine proteases. Of these classes of enzymes, a number can hydrolyze elastin and have been shown to be elevated in COPD patients (neutrophil elastase, MMP-2, 9, 12) (Culpitt *et al.*, *Am. J. Respir. Crit. Care Med. 160*, 1635-39, 1999, Shapiro, *Am. J. Crit. Care Med. 160 (5)*, S29 – S32,1999).

It is expected that in the future novel members of the existing classes of proteases and new classes of proteases will be identified that play a significant role in the damage of the extracellular lung matrix including elastin proteolysis. Novel protease targets therefore remain very attractive therapeutic targets.

CNS disorders which may be treated include brain injuries, cerebrovascular diseases and their consequences, Parkinson's disease, corticobasal degeneration, motor neuron disease, dementia, including ALS, multiple sclerosis, traumatic brain injury, stroke, post-stroke, post-traumatic brain injury, and small-vessel cerebrovascular disease. Dementias, such as Alzheimer's disease, vascular dementia, dementia with Lewy bodies, frontotemporal dementia and Parkinsonism linked to chromosome 17, frontotemporal dementias, including Pick's disease, progressive nuclear palsy, corticobasal degeneration, Huntington's disease, thalamic degeneration, Creutzfeld-Jakob dementia, HIV dementia, schizophrenia with dementia, and Korsakoff's psychosis also can be treated. Similarly, it may be possible to treat cognitive-related disorders, such as mild cognitive impairment, age-associated memory impairment, age-related cognitive decline, vascular cognitive impairment, attention deficit disorders, attention deficit hyperactivity disorders, and memory disturbances in

children with learning disabilities, by regulating the activity of human ADAM-TS-like zinc metalloprotease.

Pain that is associated with CNS disorders also can be treated by regulating the activity of human ADAM-TS-like zinc metalloprotease. Pain which can be treated includes that associated with central nervous system disorders, such as multiple sclerosis, spinal cord injury, sciatica, failed back surgery syndrome, traumatic brain injury, epilepsy, Parkinson's disease, post-stroke, and vascular lesions in the brain and spinal cord (*e.g.*, infarct, hemorrhage, vascular malformation). Non-central neuropathic pain includes that associated with post mastectomy pain, reflex sympathetic dystrophy (RSD), trigeminal neuralgiaradioculopathy, post-surgical pain, HIV/AIDS related pain, cancer pain, metabolic neuropathies (*e.g.*, diabetic neuropathy, vasculitic neuropathy secondary to connective tissue disease), paraneoplastic polyneuropathy associated, for example, with carcinoma of lung, or leukemia, or lymphoma, or carcinoma of prostate, colon or stomach, trigeminal neuralgia, cranial neuralgias, and post-herpetic neuralgia. Pain associated with cancer and cancer treatment also can be treated, as can headache pain (for example, migraine with aura, migraine without aura, and other migraine disorders), episodic and chronic tension-type headache, tension-type like headache, cluster headache, and chronic paroxysmal hemicrania.

Cardiovascular diseases include the following disorders of the heart and the vascular system: congestive heart failure, myocardial infarction, ischemic diseases of the heart, all kinds of atrial and ventricular arrhythmias, hypertensive vascular diseases, and peripheral vascular diseases.

Heart failure is defined as a pathophysiologic state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate commensurate with the requirement of the metabolizing tissue. It includes all forms of pumping failure, such as high-output and low-output, acute and chronic, right-sided or left-sided, systolic or diastolic, independent of the underlying cause.

Myocardial infarction (MI) is generally caused by an abrupt decrease in coronary blood flow that follows a thrombotic occlusion of a coronary artery previously narrowed by arteriosclerosis. MI prophylaxis (primary and secondary prevention) is included, as well as the acute treatment of MI and the prevention of complications.

Ischemic diseases are conditions in which the coronary flow is restricted resulting in a perfusion which is inadequate to meet the myocardial requirement for oxygen. This group of diseases includes stable angina, unstable angina, and asymptomatic ischemia.

Arrhythmias include all forms of atrial and ventricular tachyarrhythmias (atrial tachycardia, atrial flutter, atrial fibrillation, atrio-ventricular reentrant tachycardia, preexcitation syndrome, ventricular tachycardia, ventricular flutter, and ventricular fibrillation), as well as bradycardic forms of arrhythmias.

Vascular diseases include primary as well as all kinds of secondary arterial hypertension (renal, endocrine, neurogenic, others). The disclosed gene and its product may be used as drug targets for the treatment of hypertension as well as for the prevention of all complications.

Peripheral vascular diseases are defined as vascular diseases in which arterial and/or venous flow is reduced resulting in an imbalance between blood supply and tissue oxygen demand. It includes chronic peripheral arterial occlusive disease (PAOD), acute arterial thrombosis and embolism, inflammatory vascular disorders, Raynaud's phenomenon, and venous disorders.

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a modulating agent, an

antisense nucleic acid molecule, a specific antibody, ribozyme, or a ADAM-TS-like zinc metalloprotease polypeptide binding molecule) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

A reagent which affects ADAM-TS-like zinc metalloprotease activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce ADAM-TS-like zinc metalloprotease activity. The reagent preferably binds to an expression product of a human ADAM-TS-like zinc metalloprotease gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells that have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain, lymph nodes, and skin.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6

cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to poly-ethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a particular cell type, such as a cell-specific ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods that are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μg to about 5 μg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al. Trends in Biotechnol. 11*, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem. 263*, 621-24 (1988); Wu *et al.*, *J. Biol. Chem. 269*, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem. 266*, 338-42 (1991).

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases ADAM-TS-like zinc metalloprotease activity relative to the ADAM-TS-like zinc metalloprotease activity which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}.

Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors that can be taken into account include the severity of the disease state,

general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 μg to about 50 μg/kg, about 50 μg to about 5 mg/kg, about 100 μg to about 500 μg/kg of patient body weight, and about 200 to about 250 μg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20 μg to about 100 μg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides that express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of a ADAM-TS-like zinc metalloprotease gene or the activity of a ADAM-TS-like zinc metalloprotease polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a ADAM-TS-like zinc metalloprotease gene or the activity of a ADAM-TS-like zinc metalloprotease polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to ADAM-TS-like zinc metalloprotease-specific mRNA, quantitative RT-PCR, immunologic detection of a ADAM-TS-like zinc metalloprotease polypeptide, or measurement of ADAM-TS-like zinc metalloprotease activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

encode the enzyme. For example, differences can be determined between the cDNA or genomic sequence encoding ADAM-TS-like zinc metalloprotease in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized, for example, by high resolution gel electrophoresis. DNA fragments of different sequences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (*see, e.g.*, Myers *et al.*, *Science 230*, 1242, 1985). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (*e.g.*, Cotton *et al.*, *Proc. Natl. Acad. Sci. USA 85*, 4397-4401, 1985). Thus, the detection of a specific DNA sequence can be performed

by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes and Southern blotting of genomic DNA. In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

Altered levels of ADAM-TS-like zinc metalloprotease also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Detection of ADAM-TS-like Zinc Metalloprotease activity

The polynucleotide of SEQ ID NO: 1 is inserted into the expression vector pCEV4 and the expression vector pCEV4-ADAM-TS-like Zinc Metalloprotease activity polypeptide obtained is transfected into human embryonic kidney 293 cells. From these cells extracts are obtained and the collagenase activity is determined in an assay in which 5 µl of the cell extract is added to 45 µl of 10 mg-mll gelatin or 10 mg-mll type IV collagen (Sigma type IV) in a solution containing 50 mM Tris Cl, 145 mM NaCl, and 5 mM CaCl2 and incubated at 37 °C for 18 h. Intact collagen is precipitated with an equal volume of 50 % trichloroacetic acid, and the levels of soluble amino acids and peptides are estimated by a standard ninhydrin method (16). Activity is measured as the difference in acid-soluble amino acids and peptides between the 18-h samples and zero time controls in which 5 µl of the cell extract is added to substrate after it has been incubated at 37 °C for 18 h and immediately prior to acid precipitation. Activity is estimated by comparison with a standard curve of clostridiopeptidase A (EC 3.4.24.3; Sigma) assuming a preparation activity of 1.8 U mgl as labeled. It is shown that the polypeptide of SEQ ID NO: 2 has a ADAM-TS-like Zinc Metalloprotease activity.

EXAMPLE 2

Expression of recombinant human ADAM-TS-like zinc metalloprotease

The *Pichia pastoris* expression vector pPICZB (Invitrogen, San Diego, CA) is used to produce large quantities of recombinant human ADAM-TS-like zinc metalloprotease polypeptides in yeast. The ADAM-TS-like zinc metalloprotease-encoding DNA sequence is derived from SEQ ID NO:1. Before insertion into vector pPICZB, the DNA sequence is modified by well known methods in such a way that it contains at its 5'-end an initiation codon and at its 3'-end an enterokinase cleavage site, a His6 reporter tag and a termination codon. Moreover, at both termini recognition sequences for restriction endonucleases are added and after digestion of the multiple

cloning site of pPICZ B with the corresponding restriction enzymes the modified DNA sequence is ligated into pPICZB. This expression vector is designed for inducible expression in *Pichia pastoris*, driven by a yeast promoter. The resulting pPICZ/md-His6 vector is used to transform the yeast.

The yeast is cultivated under usual conditions in 5 liter shake flasks and the recombinantly produced protein isolated from the culture by affinity chromatography (Ni-NTA-Resin) in the presence of 8 M urea. The bound polypeptide is eluted with buffer, pH 3.5, and neutralized. Separation of the polypeptide from the His6 reporter tag is accomplished by site-specific proteolysis using enterokinase (Invitrogen, San Diego, CA) according to manufacturer's instructions. Purified human ADAM-TS-like zinc metalloprotease polypeptide is obtained.

EXAMPLE 3

Identification of test compounds that bind to ADAM-TS-like zinc metalloprotease polypeptides

Purified ADAM-TS-like zinc metalloprotease polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Human ADAM-TS-like zinc metallo-protease polypeptides comprise the amino acid sequence shown in SEQ ID NO:2. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a ADAM-TS-like zinc metalloprotease polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound that increases the fluorescence in a well by at least 15% relative to

fluorescence of a well in which a test compound is not incubated is identified as a compound which binds to a ADAM-TS-like zinc metalloprotease polypeptide.

EXAMPLE 4

Identification of a test compound which decreases ADAM-TS-like zinc metalloprotease gene expression

A test compound is administered to a culture of human cells transfected with a ADAM-TS-like zinc metalloprotease expression construct and incubated at 37 °C for 10 to 45 minutes. A culture of the same type of cells that have not been transfected is incubated for the same time without the test compound to provide a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18*, 5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and hybridized with a ^{32}P-labeled ADAM-TS-like zinc metalloprotease-specific probe at 65 ° C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NO:1. A test compound that decreases the ADAM-TS-like zinc metalloprotease-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of ADAM-TS-like zinc metalloprotease gene expression.

EXAMPLE 5

Identification of a test compound which decreases ADAM-TS-like zinc metalloprotease activity

A test compound is administered to a culture of human cells transfected with a ADAM-TS-like zinc metalloprotease expression construct and incubated at 37 °C for 10 to 45 minutes. A culture of the same type of cells that have not been transfected is incubated for the same time without the test compound to provide a negative control. Enzyme activity is measured using the method of See, *e.g.*, Black & White, *Curr. Opin. Cell Biol. 10*, 654-59, 1998.

A test compound which decreases the ADAM-TS-like zinc metalloprotease activity of the ADAM-TS-like zinc metalloprotease relative to the ADAM-TS-like zinc metalloprotease activity in the absence of the test compound is identified as an inhibitor of ADAM-TS-like zinc metalloprotease activity.

EXAMPLE 6

Tissue-specific expression of ADAM-TS-like zinc metalloprotease

The qualitative expression pattern of ADAM-TS-like zinc metalloprotease in various tissues is determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

Expression profiling of the ADAM TS-like precursor gene was done using real-time quantitative PCR (TaqMan) with RNA samples isolated from a wide range of human cells and tissues. Total RNA samples were either purchased from commercial suppliers or purified in-house. Two panels of RNAs were used for profiling: a whole body organ panel (Table 1) and a respiratory specific panel (Table 2).

Real-time quantitative PCR. This technique is a development of the kinetic analysis of PCR first described by Higuchi *et al.* (*BioTechnology 10*, 413-17, 1992; *BioTechnology 11*, 1026-30, 1993). The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies. PCR amplification is performed in the presence of an oligonucleotide probe (TaqMan probe) that is complementary to the target sequence and labeled with a fluorescent reporter dye and a quencher dye. During the extension phase of PCR, the probe is cleaved by the 5'-3' endonuclease activity of Taq DNA polymerase, releasing the fluorophore from the effect of the quenching dye (Holland *et al., Proc. Natl. Acad. Sci. U.S.A. 88*, 7276-80, 1991). Because the fluorescence emission increases in direct proportion to the amount of the specific amplified product, the exponential growth phase of PCR product can be detected and used to

SUPERSCRIPT™ II RNaseH⁻ Reverse Transcriptase (Life Technologies, Paisley, UK), 10mM dithiothreitol, 0.5mM of each dNTP, and 5μM random hexamers (PE Applied Biosystems, Warrington, Cheshire, UK) in a final volume of 20μl according to the manufacturer's protocol.

TaqMan quantitative analysis. Specific primers and probe were designed according to the recommendations of PE Applied Biosystems and are listed below:

Forward primer: 5'- TGCCCATGAGTCTGGACACA -3'

Reverse primer: 5'- CATGATGTTGCCCTCGGACT -3'

Probe: 5'-(FAM)- TCCCTTCTCCATCATGAATCATGCCAAA-3'

where FAM = 6-carboxy-fluorescein.

Quantitative PCR was performed with 10ng of reverse transcribed RNA from each sample. Each determination was done in duplicate.

The assay reaction mix was as follows: 1X final TaqMan Universal PCR Master Mix (from 2X stock) (PE Applied Biosystems, CA); 900nM forward primer; 900nM reverse primer; 200nM probe; 10ng cDNA; and water to 25μl.

Each of the following steps were carried out once: pre PCR, 2 minutes at 50°C, and 10 minutes at 95°C. The following steps were carried out 40 times: denaturation, 15 seconds at 95°C, annealing/extension, 1 minute at 60°C.

Real-time quantitative PCR was done using an ABI Prism 7700 Sequence Detector. The C_T value generated for each reaction was used to determine the initial template concentration (copy number) by interpolation from a universal standard curve. The level of expression of the target gene in each sample was calculated relative to the sample with the lowest expression of the gene. The results are shown in Figs. 10 and 11.

Table 1. Human organ RNA panel used for real-time quantitative PCR.

All samples were obtained from Clontech UK Ltd, Basingstoke, UK.

Tissue	Cat. #
Adrenal gland	Human Panel V, K4004-1
Bone marrow	Human Panel II, K4001-1
Brain	Human Panel I, K4000-1
Colon	Human Panel II, K4001-1
Heart	Human Panel III, K4002-1
Kidney	Human Panel I, K4000-1
Liver	Human Panel I, K4000-1
Lung	Human Panel I, K4000-1
Mammary gland	Human Panel III, K4002-1
Pancreas	Human Panel V, K4004-1
Prostate	Human Panel III, K4002-1
Salivary gland	Human Panel V, K4004-1
Skeletal muscle	Human Panel III, K4002-1
Small intestine	Human Panel II, K4001-1
Spleen	Human Panel II, K4001-1
Stomach	Human Panel II, K4001-1
Testis	Human Panel III, K4002-1
Thymus	Human Panel II, K4001-1
Thyroid	Human Panel V, K4004-1
Uterus	Human Panel III, K4002-1

Table 2. Human respiratory specific RNA panel used for real-time quantitative PCR.

Tissue/cell type	***Supplier, cat #***
Lung (fetal)	Takara (Japan)
Lung	Clontech, Human Panel I, K4000-1
Trachea	Clontech, Human Panel I, K4000-1
Cultured human bronchial epithelial cells	In-house
Cultured airway smooth muscle cells	In-house
Cultured small airway epithelial cells	In-house
Primary cultured alveolar type II cells	In-house
Cultured H441 cells (Clara-like)	In-house
Freshly isolated polymorphonuclear leukocytes (neutrophils)	In-house
Freshly isolated monocytes	In-house
Cultured monocytes (macrophage-like)	In-house

To demonstrate that ADAM-TS-like zinc metalloprotease is involved in CNS disorders, the following tissues are analysed as described above: fetal and adult brain, muscle, heart, lung, kidney, liver, thymus, testis, colon, placenta, trachea, pancreas, kidney, gastric mucosa, colon, liver, cerebellum, skin, cortex (Alzheimer's and normal), hypothalamus, cortex, amygdala, cerebellum, hippocampus, choroid, plexus, thalamus, and spinal cord.

EXAMPLE 7

In vivo testing of compounds/target validation

1. Pain:

Acute Pain

Acute pain is measured on a hot plate mainly in rats. Two variants of hot plate testing are used: In the classical variant animals are put on a hot surface (52 to 56 °C) and

lick a hind paw. The temperature which is reached when hind paw licking begins is a measure for pain threshold.

Compounds are tested against a vehicle treated control group. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

Persistent Pain

Persistent pain is measured with the formalin or capsaicin test, mainly in rats. A solution of 1 to 5% formalin or 10 to 100 µg capsaicin is injected into one hind paw of the experimental animal. After formalin or capsaicin application the animals show nocifensive reactions like flinching, licking and biting of the affected paw. The number of nocifensive reactions within a time frame of up to 90 minutes is a measure for intensity of pain.

Compounds are tested against a vehicle treated control group. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to formalin or capsaicin administration.

Neuropathic Pain

Neuropathic pain is induced by different variants of unilateral sciatic nerve injury mainly in rats. The operation is performed under anesthesia. The first variant of sciatic nerve injury is produced by placing loosely constrictive ligatures around the common sciatic nerve. The second variant is the tight ligation of about the half of the diameter of the common sciatic nerve. In the next variant, a group of models is used

in which tight ligations or transections are made of either the L5 and L6 spinal nerves, or the L% spinal nerve only. The fourth variant involves an axotomy of two of the three terminal branches of the sciatic nerve (tibial and common peroneal nerves) leaving the remaining sural nerve intact whereas the last variant comprises the axotomy of only the tibial branch leaving the sural and common nerves uninjured. Control animals are treated with a sham operation.

Postoperatively, the nerve injured animals develop a chronic mechanical allodynia, cold allodynioa, as well as a thermal hyperalgesia. Mechanical allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, SA, USA; Electronic von Frey System, Somedic Sales AB, Hörby, Sweden). Thermal hyperalgesia is measured by means of a radiant heat source (Plantar Test, Ugo Basile, Comerio, Italy), or by means of a cold plate of 5 to 10 °C where the nocifensive reactions of the affected hind paw are counted as a measure of pain intensity. A further test for cold induced pain is the counting of nocifensive reactions, or duration of nocifensive responses after plantar administration of acetone to the affected hind limb. Chronic pain in general is assessed by registering the circadanian rhythms in activity (Surjo and Arndt, Universität zu Köln, Cologne, Germany), and by scoring differences in gait (foot print patterns; FOOTPRINTS program, Klapdor et al., 1997. A low cost method to analyze footprint patterns. J. Neurosci. Methods 75, 49-54).

Compounds are tested against sham operated and vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

Inflammatory Pain

Inflammatory pain is induced mainly in rats by injection of 0.75 mg carrageenan or complete Freund's adjuvant into one hind paw. The animals develop an edema with mechanical allodynia as well as thermal hyperalgesia. Mechanical allodynia is

measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, SA, USA). Thermal hyperalgesia is measured by means of a radiant heat source (Plantar Test, Ugo Basile, Comerio, Italy, Paw thermal stimulator, G. Ozaki, University of California, USA). For edema measurement two methods are being used. In the first method, the animals are sacrificed and the affected hindpaws sectioned and weighed. The second method comprises differences in paw volume by measuring water displacement in a plethysmometer (Ugo Basile, Comerio, Italy).

Compounds are tested against uninflamed as well as vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

Diabetic Neuropathic Pain

Rats treated with a single intraperitoneal injection of 50 to 80 mg/kg streptozotocin develop a profound hyperglycemia and mechanical allodynia within 1 to 3 weeks. Mechanical allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, SA, USA).

Compounds are tested against diabetic and non-diabetic vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

2. Parkinson's disease

6-Hydroxydopamine (6-OH-DA) Lesion

Degeneration of the dopaminergic nigrostriatal and striatopallidal pathways is the central pathological event in Parkinson's disease. This disorder has been mimicked

experimentally in rats using single/sequential unilateral stereotaxic injections of 6-OH-DA into the medium forebrain bundle (MFB).

Male Wistar rats (Harlan Winkelmann, Germany), weighing 200±250 g at the beginning of the experiment, are used. The rats are maintained in a temperature- and humidity-controlled environment under a 12 h light/dark cycle with free access to food and water when not in experimental sessions. The following in vivo protocols are approved by the governmental authorities. All efforts are made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques.

Animals are administered pargyline on the day of surgery (Sigma, St. Louis, MO, USA; 50 mg/kg i.p.) in order to inhibit metabolism of 6-OHDA by monoamine oxidase and desmethylimipramine HCl (Sigma; 25 mg/kg i.p.) in order to prevent uptake of 6-OHDA by noradrenergic terminals. Thirty minutes later the rats are anesthetized with sodium pentobarbital (50 mg/kg) and placed in a stereotaxic frame. In order to lesion the DA nigrostriatal pathway 4 µl of 0.01% ascorbic acid-saline containing 8 µg of 6-OHDA HBr (Sigma) are injected into the left medial fore-brain bundle at a rate of 1 µl/min (2.4 mm anterior, 1.49 mm lateral, -2.7 mm ventral to Bregma and the skull surface). The needle is left in place an additional 5 min to allow diffusion to occur.

Stepping Test

Forelimb akinesia is assessed three weeks following lesion placement using a modified stepping test protocol. In brief, the animals are held by the experimenter with one hand fixing the hindlimbs and slightly raising the hind part above the surface. One paw is touching the table, and is then moved slowly sideways (5 s for 1 m), first in the forehand and then in the backhand direction. The number of adjusting steps is counted for both paws in the backhand and forehand direction of movement. The sequence of testing is right paw forehand and backhand adjusting stepping,

followed by left paw forehand and backhand directions. The test is repeated three times on three consecutive days, after an initial training period of three days prior to the first testing. Forehand adjusted stepping reveals no consistent differences between lesioned and healthy control animals. Analysis is therefore restricted to backhand adjusted stepping.

Balance Test

Balance adjustments following postural challenge are also measured during the stepping test sessions. The rats are held in the same position as described in the stepping test and, instead of being moved sideways, tilted by the experimenter towards the side of the paw touching the table. This maneuver results in loss of balance and the ability of the rats to regain balance by forelimb movements is scored on a scale ranging from 0 to 3. Score 0 is given for a normal forelimb placement. When the forelimb movement is delayed but recovery of postural balance detected, score 1 is given. Score 2 represents a clear, yet insufficient, forelimb reaction, as evidenced by muscle contraction, but lack of success in recovering balance, and score 3 is given for no reaction of movement. The test is repeated three times a day on each side for three consecutive days after an initial training period of three days prior to the first testing.

Staircase Test (Paw Reaching)

A modified version of the staircase test is used for evaluation of paw reaching behavior three weeks following primary and secondary lesion placement. Plexiglass test boxes with a central platform and a removable staircase on each side are used. The apparatus is designed such that only the paw on the same side at each staircase can be used, thus providing a measure of independent forelimb use. For each test the animals are left in the test boxes for 15 min. The double staircase is filled with 7 x 3 chow pellets (Precision food pellets, formula: P, purified rodent diet, size 45 mg; Sandown Scientific) on each side. After each test the number of pellets eaten

(successfully retrieved pellets) and the number of pellets taken (touched but dropped) for each paw and the success rate (pellets eaten/pellets taken) are counted separately. After three days of food deprivation (12 g per animal per day) the animals are tested for 11 days. Full analysis is conducted only for the last five days.

MPTP treatment

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) causes degeneration of mesencephalic dopaminergic (DAergic) neurons in rodents, non-human primates, and humans and, in so doing, reproduces many of the symptoms of Parkinson's disease. MPTP leads to a marked decrease in the levels of dopamine and its metabolites, and in the number of dopaminergic terminals in the striatum as well as severe loss of the tyrosine hydroxylase (TH)-immunoreactive cell bodies in the substantia nigra, pars compacta.

In order to obtain severe and long-lasting lesions, and to reduce mortality, animals receive single injections of MPTP, and are then tested for severity of lesion 7–10 days later. Successive MPTP injections are administered on days 1, 2 and 3. Animals receive application of 4 mg/kg MPTP hydrochloride (Sigma) in saline once daily. All injections are intraperitoneal (i.p.) and the MPTP stock solution is frozen between injections. Animals are decapitated on day 11.

Immunohistology

At the completion of behavioral experiments, all animals are anaesthetized with 3 ml thiopental (1 g/40 ml i.p., Tyrol Pharma). The mice are perfused transcardially with 0.01 M PBS (pH 7.4) for 2 min, followed by 4% paraformaldehyde (Merck) in PBS for 15 min. The brains are removed and placed in 4% paraformaldehyde for 24 h at 4 °C. For dehydration they are then transferred to a 20% sucrose (Merck) solution in 0.1 M PBS at 4 °C until they sink. The brains are frozen in methylbutan at -20 °C for 2 min and stored at -70 °C. Using a sledge microtome (mod. 3800-Frigocut, Leica),

25 µm sections are taken from the genu of the corpus callosum (AP 1.7 mm) to the hippocampus (AP 21.8 mm) and from AP 24.16 to AP 26.72. Forty-six sections are cut and stored in assorters in 0.25 M Tris buffer (pH 7.4) for immunohistochemistry.

A series of sections is processed for free-floating tyrosine hydroxylase (TH) immunohistochemistry. Following three rinses in 0.1 M PBS, endogenous peroxidase activity is quenched for 10 min in 0.3% H_2O_2 ±PBS. After rinsing in PBS, sections are preincubated in 10% normal bovine serum (Sigma) for 5 min as blocking agent and transferred to either primary anti-rat TH rabbit antiserum (dilution 1:2000).

Following overnight incubation at room temperature, sections for TH immunoreactivity are rinsed in PBS (2 x10 min) and incubated in biotinylated anti-rabbit immunoglobulin G raised in goat (dilution 1:200) (Vector) for 90 min, rinsed repeatedly and transferred to Vectastain ABC (Vector) solution for 1 h. 3,.3' -Diaminobenzidine tetrahydrochloride (DAB; Sigma) in 0.1 M PBS, supplemented with 0.005% H_2O_2 , serves as chromogen in the subsequent visualization reaction. Sections are mounted on to gelatin-coated slides, left to dry overnight, counter-stained with hematoxylin dehydrated in ascending alcohol concentrations and cleared in butylacetate. Coverslips are mounted on entellan.

Rotarod Test

We use a modification of the procedure described by Rozas and Labandeira-Garcia (1997), with a CR-1 Rotamex system (Columbus Instruments, Columbus, OH) comprising an IBM-compatible personal computer, a CIO-24 data acquisition card, a control unit, and a four-lane rotarod unit. The rotarod unit consists of a rotating spindle (diameter 7.3 cm) and individual compartments for each mouse. The system software allows preprogramming of session protocols with varying rotational speeds (0–80 rpm). Infrared beams are used to detect when a mouse has fallen onto the base grid beneath the rotarod. The system logs the fall as the end of the experiment for that mouse, and the total time on the rotarod, as well as the time of the fall and all the

set-up parameters, are recorded. The system also allows a weak current to be passed through the base grid, to aid training.

3. Dementia

The object recognition task

The object recognition task has been designed to assess the effects of experimental manipulations on the cognitive performance of rodents. A rat is placed in an open field, in which two identical objects are present. The rats inspects both objects during the first trial of the object recognition task. In a second trial, after a retention interval of for example 24 hours, one of the two objects used in the first trial, the 'familiar' object, and a novel object are placed in the open field. The inspection time at each of the objects is registered. The basic measures in the OR task is the time spent by a rat exploring the two object the second trial. Good retention is reflected by higher exploration times towards the novel than the 'familiar' object.

Administration of the putative cognition enhancer prior to the first trial predominantly allows assessment of the effects on acquisition, and eventually on consolidation processes. Administration of the testing compound after the first trial allows to assess the effects on consolidation processes, whereas administration before the second trial allows to measure effects on retrieval processes.

The passive avoidance task

The passive avoidance task assesses memory performance in rats and mice. The inhibitory avoidance apparatus consists of a two-compartment box with a light compartment and a dark compartment. The two compartments are separated by a guillotine door that can be operated by the experimenter. A threshold of 2 cm separates the two compartments when the guillotine door is raised. When the door is open, the illumination in the dark compartment is about 2 lux. The light intensity is about 500 lux at the center of the floor of the light compartment.

enter the dark compartment within a few seconds.

In the shock session the guillotine door between the compartments is lowered as soon as the rat has entered the dark compartment with its four paws, and a scrambled 1 mA footshock is administered for 2 sec. The rat is removed from the apparatus and put back into its home cage. The procedure during the retention session is identical to that of the habituation sessions.

The step-through latency, that is the first latency of entering the dark compartment (in sec.) during the retention session is an index of the memory performance of the animal; the longer the latency to enter the dark compartment, the better the retention is. A testing compound in given half an hour before the shock session, together with 1 $mg*kg^{-1}$ scopolamine. Scopolamine impairs the memory performance during the retention session 24 hours later. If the test compound increases the enter latency compared with the scopolamine-treated controls, is likely to possess cognition enhancing potential.

The Morris water escape task

The Morris water escape task measures spatial orientation learning in rodents. It is a test system that has extensively been used to investigate the effects of putative therapeutic on the cognitive functions of rats and mice. The performance of an animal is assessed in a circular water tank with an escape platform that is submerged about 1 cm below the surface of the water. The escape platform is not visible for an

animal swimming in the water tank. Abundant extra-maze cues are provided by the furniture in the room, including desks, computer equipment, a second water tank, the presence of the experimenter, and by a radio on a shelf that is playing softly.

The animals receive four trials during five daily acquisition sessions. A trial is started by placing an animal into the pool, facing the wall of the tank. Each of four starting positions in the quadrants north, east, south, and west is used once in a series of four trials; their order is randomized. The escape platform is always in the same position. A trial is terminated as soon as the animal had climbs onto the escape platform or when 90 seconds have elapsed, whichever event occurs first. The animal is allowed to stay on the platform for 30 seconds. Then it is taken from the platform and the next trial is started. If an animal did not find the platform within 90 seconds it is put on the platform by the experimenter and is allowed to stay there for 30 seconds. After the fourth trial of the fifth daily session, an additional trial is given as a probe trial: the platform is removed, and the time the animal spends in the four quadrants is measured for 30 or 60 seconds. In the probe trial, all animals start from the same start position, opposite to the quadrant where the escape platform had been positioned during acquisition.

Four different measures are taken to evaluate the performance of an animal during acquisition training: escape latency, traveled distance, distance to platform, and swimming speed. The following measures are evaluated for the probe trial: time (s) in quadrants and traveled distance (cm) in the four quadrants. The probe trial provides additional information about how well an animal learned the position of the escape platform. If an animal spends more time and swims a longer distance in the quadrant where the platform had been positioned during the acquisition sessions than in any other quadrant, one concludes that the platform position has been learned well.

In order to assess the effects of putative cognition enhancing compounds, rats or mice with specific brain lesions which impair cognitive functions, or animals treated

by lowering the guillotine door. After the mouse has been released from the start arm, it will negotiate the maze, eventually enter the open goal arm, and return to the start position, where it will be confined for 5 seconds, by lowering the guillotine door. Then, the animal can choose freely between the left and right goal arm (all guillotine-doors opened) during 14 'free choice' trials. As soon a the mouse has entered one goal arm, the other one is closed. The mouse eventually returns to the start arm and is free to visit whichever go alarm it wants after having been confined to the start arm for 5 seconds. After completion of 14 free choice trials in one session, the animal is removed from the maze. During training, the animal is never handled.

The percent alternations out of 14 trials is calculated. This percentage and the total time needed to complete the first forced trial and the subsequent 14 free choice trials (in s) is analyzed. Cognitive deficits are usually induced by an injection of scopolamine, 30 min before the start of the training session. Scopolamine reduced the per-cent alternations to chance level, or below. A cognition enhancer, which is always administered before the training session, will at least partially, antagonize the scopolamine-induced reduction in the spontaneous alternation rate.

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d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes a ADAM-TS-like zinc metalloprotease polypeptide; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and encodes a ADAM-TS-like zinc metalloprotease polypeptide.

2. An expression vector containing any polynucleotide of claim 1.

3. A host cell containing the expression vector of claim 2.

4. A substantially purified ADAM-TS-like zinc metalloprotease polypeptide encoded by a polynucleotide of claim 1.

5. A method for producing a ADAM-TS-like zinc metalloprotease polypeptide, wherein the method comprises the following steps:

a) culturing the host cell of claim 3 under conditions suitable for the expression of the ADAM-TS-like zinc metalloprotease polypeptide; and

b) recovering the ADAM-TS-like zinc metalloprotease polypeptide from the host cell culture.

6. A method for detection of a polynucleotide encoding a ADAM-TS-like zinc metalloprotease polypeptide in a biological sample comprising the following steps:

a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b) detecting said hybridization complex.

7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.

8. A method for the detection of a polynucleotide of claim 1 or a ADAM-TS-like zinc metalloprotease polypeptide of claim 4 comprising the steps of:
contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the ADAM-TS-like zinc metalloprotease polypeptide.

9. A diagnostic kit for conducting the method of any one of claims 6 to 8.

10. A method of screening for agents which decrease the activity of a ADAM-TS-like zinc metalloprotease, comprising the steps of:
contacting a test compound with any ADAM-TS-like zinc metalloprotease polypeptide encoded by any polynucleotide of claim1;
detecting binding of the test compound to the ADAM-TS-like zinc metalloprotease polypeptide, wherein a test compound which binds to the

metalloprotease activity is identified as a potential therapeutic agent for increasing the activity of the ADAM-TS-like zinc metalloprotease, and wherein a test compound which decreases the ADAM-TS-like zinc metalloprotease activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the ADAM-TS-like zinc metalloprotease.

12. A method of screening for agents which decrease the activity of a ADAM-TS-like zinc metalloprotease, comprising the steps of:

contacting a test compound with any polynucleotide of claim 1 and detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of ADAM-TS-like zinc metalloprotease.

13. A method of reducing the activity of ADAM-TS-like zinc metalloprotease, comprising the steps of:

contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any ADAM-TS-like zinc metalloprotease polypeptide of claim 4, whereby the activity of ADAM-TS-like zinc metalloprotease is reduced.

14. A reagent that modulates the activity of a ADAM-TS-like zinc metalloprotease polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claim 10 to 12.

15. A pharmaceutical composition, comprising:
the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.

16. Use of the expression vector of claim 2 or the reagent of claim 14 in the preparation of a medicament for modulating the activity of a ADAM-TS-like zinc metalloprotease in a disease.

17. Use of claim 16 wherein the disease is COPD, a CNS disorder, or a cardiovascular disorders.

18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.

19. The cDNA of claim 18 which comprises SEQ ID NO:1.

20. The cDNA of claim 18 which consists of SEQ ID NO:1.

21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.

22. The expression vector of claim 21 wherein the polynucleotide consists of SEQ ID NO:1.

23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.

24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO:1.

25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.

26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NO:2.

27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO:2.

28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising the steps of:
culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and
isolating the polypeptide.

29. The method of claim 28 wherein the expression vector comprises SEQ ID NO:1.

30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising the steps of:
hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO:1 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and
detecting the hybridization complex.

31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.

32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising:
a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO:1; and
instructions for the method of claim 30.

33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising the steps of:
contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and
detecting the reagent-polypeptide complex.

34. The method of claim 33 wherein the reagent is an antibody.

35. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising:
an antibody which specifically binds to the polypeptide; and
instructions for the method of claim 33.

36. A method of screening for agents which can modulate the activity of a human ADAM-TS-like zinc metalloprotease, comprising the steps of:
contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO:2 and (2) the amino acid sequence shown in SEQ ID NO:2; and
detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the human ADAM-TS-like zinc metalloprotease.

37. The method of claim 36 wherein the step of contacting is in a cell.

38. The method of claim 36 wherein the cell is *in vitro*.

39. The method of claim 36 wherein the step of contacting is in a cell-free system.

40. The method of claim 36 wherein the polypeptide comprises a detectable label.

41. The method of claim 36 wherein the test compound comprises a detectable label.

42. The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.

43. The method of claim 36 wherein the polypeptide is bound to a solid support.

44. The method of claim 36 wherein the test compound is bound to a solid support.

45. A method of screening for agents which modulate an activity of a human ADAM-TS-like zinc metalloprotease, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO:2 and (2) the amino acid sequence shown in SEQ ID NO:2; and

detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human ADAM-TS-like zinc metalloprotease, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human ADAM-TS-like zinc metalloprotease.

46. The method of claim 45 wherein the step of contacting is in a cell.

47. The method of claim 45 wherein the cell is *in vitro*.

48. The method of claim 45 wherein the step of contacting is in a cell-free system.

49. A method of screening for agents which modulate an activity of a human ADAM-TS-like zinc metalloprotease, comprising the steps of:
contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO:1; and
detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human ADAM-TS-like zinc metalloprotease.

50. The method of claim 49 wherein the product is a polypeptide.

51. The method of claim 49 wherein the product is RNA.

52. A method of reducing activity of a human ADAM-TS-like zinc metalloprotease, comprising the step of:
contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO:1, whereby the activity of a human ADAM-TS-like zinc metalloprotease is reduced.

53. The method of claim 52 wherein the product is a polypeptide.

54. The method of claim 53 wherein the reagent is an antibody.

55. The method of claim 52 wherein the product is RNA.

56. The method of claim 55 wherein the reagent is an antisense oligonucleotide.

57. The method of claim 56 wherein the reagent is a ribozyme.

58. The method of claim 52 wherein the cell is *in vitro*.

59. The method of claim 52 wherein the cell is *in vivo*.

60. A pharmaceutical composition, comprising:
a reagent which specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2; and
a pharmaceutically acceptable carrier.

61. The pharmaceutical composition of claim 60 wherein the reagent is an antibody.

62. A pharmaceutical composition, comprising:
a reagent which specifically binds to a product of a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO:1; and
a pharmaceutically acceptable carrier.

63. The pharmaceutical composition of claim 62 wherein the reagent is a ribozyme.

64. The pharmaceutical composition of claim 62 wherein the reagent is an antisense oligonucleotide.

65. The pharmaceutical composition of claim 62 wherein the reagent is an antibody.

66. A pharmaceutical composition, comprising:

an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2; and
a pharmaceutically acceptable carrier.

67. The pharmaceutical composition of claim 66 wherein the expression vector comprises SEQ ID NO:1.

68. A method of treating a ADAM-TS-like zinc metalloprotease dysfunction related disease, wherein the disease is selected from COPD, a CNS disorder, or a cardiovascular disorder comprising the step of:
administering to a patient in need thereof a therapeutically effective dose of a reagent that modulates a function of a human ADAM-TS-like zinc metalloprotease, whereby symptoms of the ADAM-TS-like zinc metalloprotease dysfunction related disease are ameliorated.

69. The method of claim 68 wherein the reagent is identified by the method of claim 36.

70. The method of claim 68 wherein the reagent is identified by the method of claim 45.

71. The method of claim 68 wherein the reagent is identified by the method of claim 49.

Fig. 1

aatgaagaac tgaacgtgga gaccttggtg gtggtcgaca
aaaagatgat gcaaaaccat ggccatgaaa atatcaccac
ctacgtgctc acgatactca acatggtatc tgctttattc
aaagatggaa caataggagg aaacatcaac attgcaattg
taggtctgat tcttctagaa gatgaacagc caggactggt
gataagtcac cacgcagacc acaccttaag tagcttctgc
cagtggcagt ctggattgat ggggaaagat gggactcgtc
atgaccacgc catcttactg actggtctgg atatatgttc
ctggaagaat gagccctgtg acactttggg atttgcaccc
ataagtggaa tgtgtagtaa atatcgcagc tgcacgatta
atgaagatac aggtcttgga ctggccttca ccattgccca
tgagtctgga cacaactttg gcatgattca tgatggagaa
gggaacatgt gcaaaaagtc cgagggcaac atcatgtccc
ctacattggc aggacgcaat ggagtcttct cctggtcacc
ctgcagccgc cagtatctac acaaatttct aagcaccgct
caagctatct gccttgctga tcagccaaag cctgtgaagg
aatacaagta tcctgagaaa ttgccaggag aattatatga
tgcaaacaca cagtgcaagt ggcagttcgg agagaaagcc
aagctctgca tgctggactt taaaaaggca accctgtggt
gccatcgtat tggaaggaaa tgtgagacta aatttatgcc
agcagcagaa ggcacaattt gtgggcatga catgtggtgc
cggggaggac agtgtgtgaa atatggtgat gaaggcccca
agcccaccca tggccactgg tcggactggt cttcttggtc
cccatgctcc aggacctgcg gaggggggagt atctcatagg
agtcgcctct gcaccaaccc caagccatcg catggaggga
agttctgtga gggctccact cgcactctga agctctgcaa
cagtcagaaa tgtccccggg acagtgttga cttccgtgct
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ggcactacaa gtggaagcct tacactcaag tagaagatca
ggacttatgc aaactctact gtatcgcaga aggatttgat
ttcttctttt ctttgtcaaa taaagtcaaa gatgggactc
catgctcgga ggatagccgt aatgtttgta tagatgggat
atgtgagaga gttggatgtg acaatgtcct tggatctgat
gctgttgaag acgtctgtgg ggtgtgtaac gggaataact
cagcctgcac gattcacagg ggtctctaca ccaagcacca
ccacaccaac cagtattatc acatggtcac cattccttct
ggagcccgga gtatccgcat ctatgaaatg aacgtctcta
cctcctacat ttctgtgcgc aatgccctca gaaggtacta
cctgaatggg cactggaccg tggactggcc cggccggtac
aaattttcgg gcactacttt cgactacaga cggtcctata
atgagcccga gaacttaatc gctactggac caaccaacga
gacactgatt gtggaggtaa agtccagc

Fig. 2

NEELNVETLV VVDKKMMQNH GHENITTYVL TILNMVSALF
KDGTIGGNIN IAIVGLILLE DEQPGLVISH HADHTLSSFC
QWQSGLMGKD GTRHDHAILL TGLDICSWKN EPCDTLGFAP
ISGMCSKYRS CTINEDTGLG LAFTIAHESG HNFGMIHDGE
GNMCKKSEGN IMSPTLAGRN GVFSWSPCSR QYLHKFLSTA
QAICLADQPK PVKEYKYPEK LPGELYDANT QCKWQFGEKA
KLCMLDFKKA TLWCHRIGRK CETKFMPAAE GTICGHDMWC
RGGQCVKYGD EGPKPTHGHW SDWSSWSPCS RTCGGGVSHR
SRLCTNPKPS HGGKFCEGST RTLKLCNSQK CPRDSVDFRA
AQCAEHNSRR FRGRHYKWKP YTQVEDQDLC KLYCIAEGFD
FFFSLSNKVK DGTPCSEDSR NVCIDGICER VGCDNVLGSD
AVEDVCGVCN GNNSACTIHR GLYTKHHHTN QYYHMVTIPS
GARSIRIYEM NVSTSYISVR NALRRYYLNG HWTVDWPGRY
KFSGTTFDYR RSYNEPENLI ATGPTNETLI VEVKSS

Fig. 3

mgdvqraarsrgslsahmllllllasitmllcargahgrpteedeelvlpsler
apghdstttrlrldafgqqlhlklqpdsgflapgftlqtvgrspgseaqhldp
tgdlahcfysgtvngdpgsaaalslcegvrgafylqgeeffiqpapgvaterl
apavpeeessarpqfhilrrrrrgsggakcgvmddetlptsdsrpesqntrnq
wpvrdptpqdagkpsgpgsirkkrfvsspryvetmlvadqsmadfhgsglkhy
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rsfsplkepltiqvlmvghalrpkikftyfmkkktesfnaiptfsewvieewg
ecsktcgsgwqrrvvqcrdinghpasecakevkpastrpcadlpcphwqvgdw
spcsktcgkgykkrtlkcvshdggvlsnescdplkkpkhyidfctltqcs

Fig. 4

ccggttcctgccatgcccggcggccccagtccccgcagccccgcgcctttgct
gcgcccccctcctcctgctcctctgcgctctggctcccggcgcccccggacccg
caccaggacgtgcaaccgagggccgggcggcactggacatcgtgcacccggtt
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agcggcagtgcacgcagcctacgcccaaatacaaaggcagatactgtgtgggt
gagcgcaagcgcttccgcctctgcaacctgcaggcctgccctgctggccgccc
ctccttccgccacgtccagtgcagccactttgacgctatgctctacaagggcc
agctgcacacatgggtgcccgtggtcaatgacgtgaacccctgcgagctgcac
tgccggcccgcgaatgagtactttgccaagaagctgcgggacgccgtggtcga
tggcacccccctgctaccaggtccgagccagccgggacctctgcatcaacggca
tctgtaagaacgtgggctgtgacttcgagattgactccggtgctatggaggac
cgctgtggtgtgtgccacggcaacggctccacctgccacaccgtgagcgggac
cttcgaggaggccgagggtctggggtatgtggatgtggggctgatcccagcgg
gcgcacgcgagatccgcatccaagaggttgccgaggctgccaacttcctggca
ctgcggagcgaggacccggagaagtacttcctcaatggtggctggaccatcca

Fig. 4 (continued)

gtggaacggggactaccaggtggcagggaccaccttcacatacgcacgcaggg
gcaactgggagaacctcacgtccccgggtcccaccaaggagcctgtctggatc
caggtgcctgcctcccgtggcccaggcgggggagcagaggcggagtccccag
gcccagcaccctccatggcaggtctcgtcctggaggagtgagccctggttcag
tcacagagcctggctctgagccaggccctcctgctgcggcctctacctcagtt
tccccatctttaaaatggcccaatcttgtagctgcagttcacagaggtggctg
gggtcaagctcctttaggactgggtggatggagaagacaccttgtgctcatgg
gcccccgcctgcccacccagctgctgttccaggagagcaaccctggggtgcac
tacgagtacaccatccacagggaggcaggtggccacgacgaggtcccgccgcc
cgtgttctcctggcattatgggccctggaccaagtgcacagtcacctgcggca
gaggtgagaagtggggcaggcacagccccacctgcaggggcttagtgtctgga
cagggacactggcttcagctcccagctcactgctgggccaccacgggtttgga
agtttgcttctctgagcctcagttctccatctgtgagatgaggctagcgattg
ccctgtgtcccaggcccgctgggagggtacatggatgaggcaggtgggtgctg
gctcgcggcgcatgttcagtgtgctccagctcttggcgttctccctccagggg
acacagctccccctcgatagaccagtccagtggcccctcaccacactgactta
tttccctaaactatttataaaaagtagggcaatttcattaactctgactctt

Fig. 5

BLASTP - alignment of 367_protein against swissnew|P97857|ATS1_MOUSE

ADAM-TS 1 PRECURSOR (EC 3.4.24.-) (A DISINTEGRIN AND METALLOPROTEINASE WITH THROMBOSPONDIN MOTIFS 1) (ADAMTS-1) (ADAM-TS1).//:swiss|P97857|ATS1_MOUSE ADAM-TS 1 PRECURSOR (EC 3.4.24.-) (A DISINTEGRIN AND METALLOPROTEINASE WITH THROMBOSPONDIN MOTIFS 1) (ADAMTS-1) (ADAM-TS1).//:trembl|AB001735|AB001735_1
product: "ADAMTS-1"; Mus musculus DNA for ADAMTS-1, complete cds.
//:pironly|T00017|T00017 gene ADAMTS-1 protein - mouse//:gp|AB001735|2809057
product: "ADAMTS-1"; Mus musculus DNA for ADAMTS-1, complete cds.

This hit is scoring at : 5e-105 (expectation value)
Alignment length (overlap) : 575
Identities : 38 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb

```
Q:       6 VETLVVVDKKMMQNHGHENITTYVLTILNMVSALFKDGTIGGNINIAIVGLILLEDEQPG
           VET::V.D:.M.. HG ..:. Y:LT:.:::.:..:K. :I .:I:::V :::: :EQ.G
H:     244 VETMLVADQSMADFHG-SGLKHYLLTLFSVAARFYKHPSIRNSISLVVVKILVIYEEQKG

           LVISHHADHTLSSFCQWQ---SGLMGKDGTRHDHAILLTGLDICSWKNEPCDTLGFAPIS
             :::.:A  TL.:FC.WQ    :. ..:D ..:D AIL.T  D:C.  :..CDTLG.A.:.
           PEVTSNAALTLRNFCNWQKQHNSPSDRDPEHYDTAILFTRQDLCG--SHTCDTLGMADVG
```


WO 02/057461 PCT/EP02/00123

Fig. 5 (continued)

H: Zn-binding E: active site

```
GMCSKYRSCTINEDTGLGLAFTIAHESGHNFGMIHDGEGNMCKKSEG-----NIMSPTLA
 :C.. RSC:: ED.GL .AFT.AHE GH F.M HD :.. C.. .G      ::M:..L:
TVCDPSRSCSVIEDDGLQAAFTTAHELGHVFNMPHD-DAKHCASLNGVTGDSHLMASMLS

GRNGVFSWSPCSRQYLHKFLSTAQAICLADQPKPVKEYKYPEKLPGELYDANTQCKWQFG
. :   .WSPCS...: .FL..... CL.D  KP ...K.P..LPG.LYDAN.QC::.FG
SLDHSQPWSPCSAYMVTSFLDNGHGECLMD--KPQNPIKLPSDLPGTLYDANRQCQFTFG

EKAKLCMLDFKKAT-LWCHRIGRK---CETKFMPAAEGTICGHDMWCRGGQCVKYGD--E
E::K C    ...T LWC  ..      C:TK..P A:GT CG...WC .G:CV.  D  .
EESKHCPDAASTCTTLWCTGTSGGLLVCQTKHFPWADGTSCGEGKWCVSGKCVNKTDMKH

GPKPTHGHWSDWSSWSPCSRTCGGGVSHRSRLCTNPKPSHGGKFCEGSTRTLKLCNSQKC
 ..P.HG.W..W..W..CSRTCGGGV.:..R C.NP P.:GGK:CEG.. ..: CN :.C
FATPVHGSWGPWGPWGDCSRTCGGGVQYTMRECDNPVPKNGGKYCEGKRVRYRSCNIEDC

P-RDSVDFRAAQCAEHN--SRRFRGRH--YKWKP-YTQVEDQDLCKLYCIAEGFDFFFSL
P .:. .FR..QC..HN  S:.  G..  .:W.P Y. V..:D CKL C A:G..:FF L
PDNNGKTFREEQCEAHNEFSKASFGNEPTVEWTPKYAGVSPKDRCKLTCEAKGIGYFFVL
```

Fig. 5 (continued)

```
SNKVKDGTPCSEDSRNVCIDGICERVGCDNVLGSDAVEDVCGVCNGNNSACTIHRGLYTK
. KV DGTPCS.DS.:VC:.G C :.GCD.:::.S..  D CGVC.GN.S.C.  .G:.T.
QPKVVDGTPCSPDSTSVCVQGQCVKAGCDRIIDSKKKFDKCGVCGGNGSTCKKMSGIVTS
HHHTNQYYH-MVTIPSGARSIRIYEMNV-----STSYISVRNALRRYYLNGHWTVDWPGR
   T.. YH :VTIP:GA.:I.: ..N      : S::::R A  .Y.LNG::T:.   :
---TRPGYHDIVTIPAGATNIEVKHRNQRGSRNNGSFLAIRAADGTYILNGNFTLSTLEQ

-YKFSGTTFDYRRSYNEPENLIATGPTNETLIVEV     553
 ..:.GT.. Y. S  . E.: : .P..E.L.::V
DLTYKGTVLRYSGSSAALERIRSFSPLKEPLTIQV     809
```

Fig. 6

BLASTP - alignment of 367_protein against trembl|AF140675|AF140675_1

gene: "ADAMTS7"; product: "zinc metalloprotease ADAMTS7"; Homo sapiens zinc metalloprotease ADAMTS7 (ADAMTS7) mRNA, complete cds. //:gp|AF140675|5923788 gene: "ADAMTS7"; product: "zinc metalloprotease ADAMTS7"; Homo sapiens zinc metalloprotease ADAMTS7 (ADAMTS7) mRNA, complete cds.

This hit is scoring at : 5e-136 (expectation value)
Alignment length (overlap) : 565
Identities : 44 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb

```
Q:       1 NEELNVETLVVVDKKMMQNHGHENITTYVLTILNMVSALFKDGTIGGNINIAIVGLILLE
             ::E  VETLVV.D.KM:: HG....:.:YVLTI:NMV:.LF.D :IG. I:I.IV L:LLE
H:     239 SKEKWVETLVVADAKMVEYHGQPQVESYVLTIMNMVAGLFHDPSIGNPIHITIVRLVLLE

             DEQPGLVISHHADHTLSSFCQWQSGLMGKDGTR---HDHAILLTGLDICSWKNEPCDTLG
             DE:..L I:HHAD:TL.SFC:WQ..:  K....   HD AILLT  D:C: .N.PC:TLG
             DEEEDLKITHHADNTLKSFCKWQKSINMKGDAHPLHHDTAILLTRKDLCAAMNRPCETLG

             FAPISGMCSKYRSCTINEDTGLGLAFTIAHESGHNFGMIHDGEGNMCKK--SEGNIMSPT
             .: ::GMC..:RSC:INEDTGL LAFT:AHE GH:FG: HDG.GN C:.  ..  IMSP.
             LSHVAGMCQPHRSCSINEDTGLPLAFTVAHELGHSFGIQHDGSGNDCEPVGKRPFIMSPQ
```

Fig. 6 (continued)

```
LAGRNGVFSWSPCSRQYLHKFLSTAQAICLADQPKPVKEYKYPEKLPGELYDANTQCKWQ
L.    . .:WS CSRQY: :FL... .:CL D.P.. . ..:P.  PG LYD.: QC: Q
LLYDAAPLTWSRCSRQYITRFLDRGWGLCL-DDPPAKDIIDFPSVPPGVLYDVSHQCRLQ

FGEKAKLCM-LDFKKATLWCHRIGRKCETKFMPAAEGTICGHDMWCRGGQCVKYGDEGPK
:G. :..C   :D    TLWC .:G..C.:K. .A.:GT CG.:.WC .G:CV..G . P:
YGAYSAFCEDMDNVCHTLWC-SVGTTCHSKLDAAVDGTRCGENKWCLSGECVPVGFR-PE

PTHGHWSDWSSWSPCSRTCGGGVSHRSRLCTNPKPSHGGKFCEGSTRTLKLCNSQKCPRD
...G WS.WS:WS CSR:CG GV....R CT.P.P.: G::C G..:..:LCN Q.CP..
AVDGGWSGWSAWSICSRSCGMGVQSAERQCTQPTPKYKGRYCVGERKRFRLCNLQACPAG

SVDFRAAQCAEHNSRRFRGRHYKWKPYTQVEDQDLCKLYCIAEGFDFFFSLSNKVKDGTP
. .FR .QC:..:::. ::G: :.W P.  V.D : C:L:C ...  F  .L.:.V DGTP
RPSFRHVQCSHFDAMLYKGQLHTWVPV--VNDVNPCELHCRPANEYFAKKLRDAVVDGTP

CSE--DSRNVCIDGICERVGCDNVLGSDAVEDVCGVCNGNNSACTIHRGLYTKHHHTNQY
C :   SR::CI:GIC:.VGCD  :.S.A:ED CGVC:GN.S.C . .G.:.: . .. Y
CYQVRASRDLCINGICKNVGCDFEIDSGAMEDRCGVCHGNGSTCHTVSGTFEEAEGLG-Y

YHMVTIPSGARSIRIYEMNVSTSYISVRNA-LRRYYLNGHWTVDWPGRYKFSGTTFDYRR
..: .IP:GAR.IRI.E:  :.:::::R:.  .:Y:LNG WT:.W G Y:.:GTTF.Y.R
VDVGLIPAGAREIRIQEVAEAANFLALRSEDPEKYFLNGGWTIQWNGDYQVAGTTFTYAR

SYNEPENLIATGPTNETLIVEVKSS     556
. N  ENL.:.GPT.E.: ::V.:S
RGNW-ENLTSPGPTKEPVWIQVPAS     796
```

Fig. 7

BLASTP - alignment of 367_protein against pdb|1ATL|1ATL-A

atrolysin c(hemorrhagic toxin c, form d)//:pdb|1HTD|1HTD-A atrolysin c(hemorrhagic toxin c, form d)

This hit is scoring at : 4e-13 (expectation value)
Alignment length (overlap) : 211
Identities : 29 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb

```
Q:      6 VETLVVVDKKMMQNHGHE--NITTYVLTILNMVSALFKDGTIGGNINIAIVGLILLEDEQ
          :E.:VV.D.::...:..:  .I.T V .I:N.:::.:.:.    NI:::::..L : .:E.
H:      6 IELVVVADHRVFMKYNSDLNTIRTRVHEIVNFINGFYRSL----NIHVSLTDLEIWSNED

          PGLVISHHADHTLSSFCQW-QSGLMGKDGTRHDHAILLTGLDICSWKNEPCDTLGFAPIS
          .  : S .:D TL::F.:W ::.L:.:..  HD:A LLT.::: .       :TLG.AP:.
          QINIQSASSD-TLNAFAEWRETDLLNRKS--HDNAQLLTAIELDE------ETLGLAPLG

          GMCSKYRSCTINED-.--TGLGLAFTIAHESGHNFGMIHDGEGNMCKKSEGNIMSPTLA-G
           MC..  S. I :D   ..L :..T:AHE GHN.GM HDG: :..: :.  IM.P L. G
          TMCDPKLSIGIVQDHSPINLLMGVTMAHELGHNLGMEHDGK-DCLRGASLCIMRPGLTKG

          RNGVFSWSPCSRQYLHKFLSTAQAICLADQP     209
          R:  :.:S. S..Y..:FL.. :. C:.::P
          RS--YEFSDDSMHYYERFLKQYKPQCILNKP     200
```

Fig. 8

HMMPFAM - alignment of 367_protein against pfam|hmm|

Reprolysin (M12B) family zinc metalloprote

This hit is scoring at : 86.5 E=5.3e-22
Scoring matrix : BLOSUM62 (used to infer consensus pa

```
Q:        6 --VETLVVVDKKMMQNHG--HENITTYVLTILNMVSALFKDgt
              :E.::VVD. M...:G   ..I.. V .I:N:V:.:::.
H:        1 rYiELvIVvDhgmytkygsdlnkirqrVhqivNlvNeiYrp.

            --DEQPgLVISHHADHTLSSFCQW-QSGLMGKdgTRHDHAILL
              D .. : :...A:.TL.SF :W ::.L: :   ..HD:A LL
            WsdgDk.InvqsdandTLhsFgeWRetdLlkr..ksHDnAqLL

            FAPISGMCSKYRSCTINEDT---GLGLAFTIAHESGHNFGMIH
             A :.GMCS. RS. : :D      L :A.T:AHE GHN.GM.H
            aAyvggmCspkrSvGVvqdhspivllvAvtMAHELGHNLGmtH

            MSPTlAGRNGVF-SWSPCSRQYLHKFLSTAQAICLADQP
            M:P. A..:     .:S CS:. ..KFL:.:.:. CL.::P
            Mnpv.assspskKkFSnCSkddyqkFltkqkpqCLlNkP
```

Fig. 9

HMMPFAM - alignment of 367_protein against pfam|hmm|tsp_1

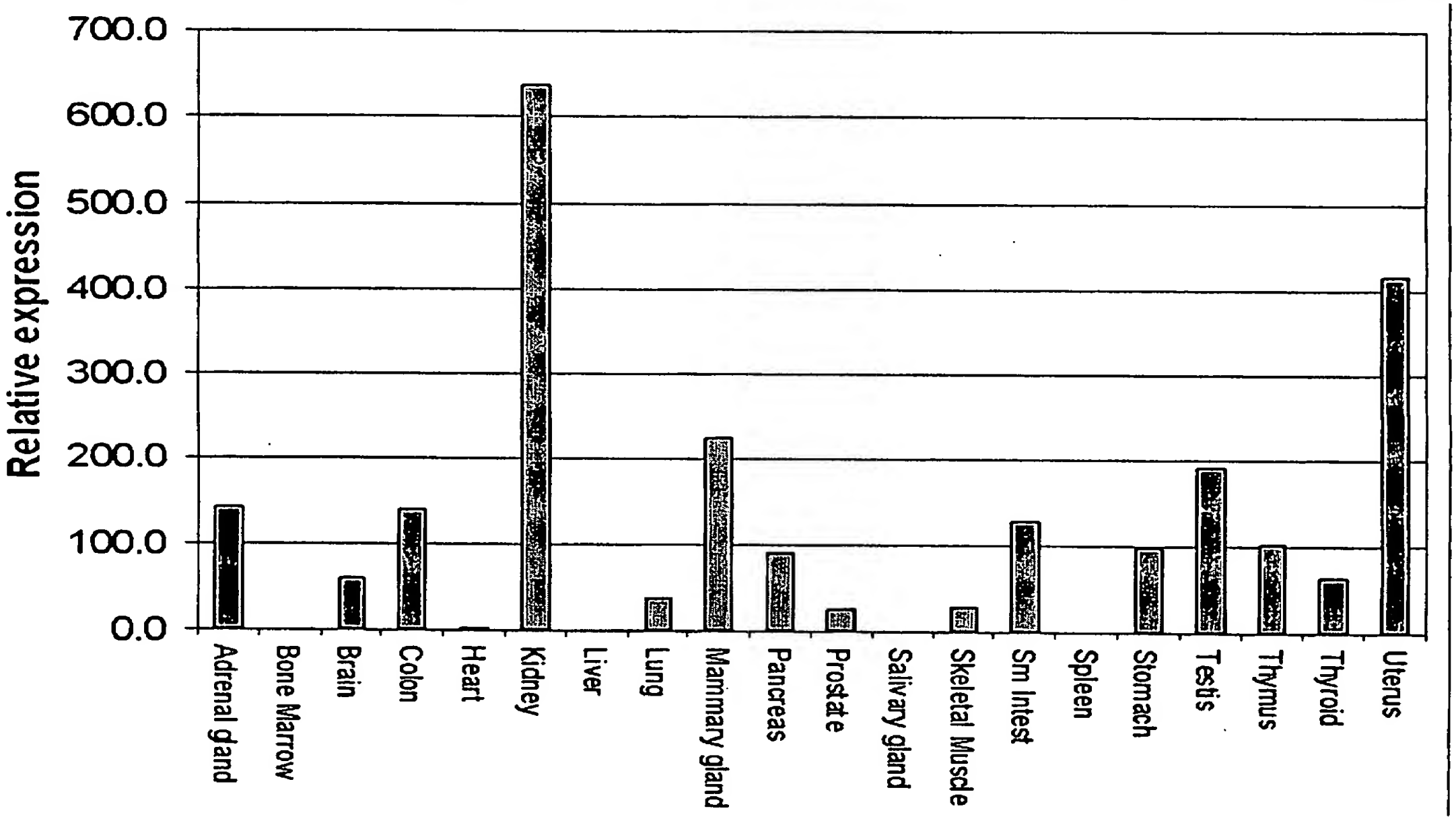
Thrombospondin type 1 domain

This hit is scoring at : 65.9 E=8.6e-16

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:    301 SDWSSWSPCSRTCGGGVSHRSRLCTNPKPS-HGGKFCEGSTRT-LKLC-NSQKC    351
          S.WS.WSPCS TCG G:. R.R.C.:P.P. .GGK C.G..:. .:.C  ..KC
H:      1 spWseWSpCSVTCGkGirtRqRtcnspaPqkkggkpCtgdaqeEteaCdmmdkC     54
```

Fig. 10 Relative expression of ADAM TS-like mRNA in various human tissu



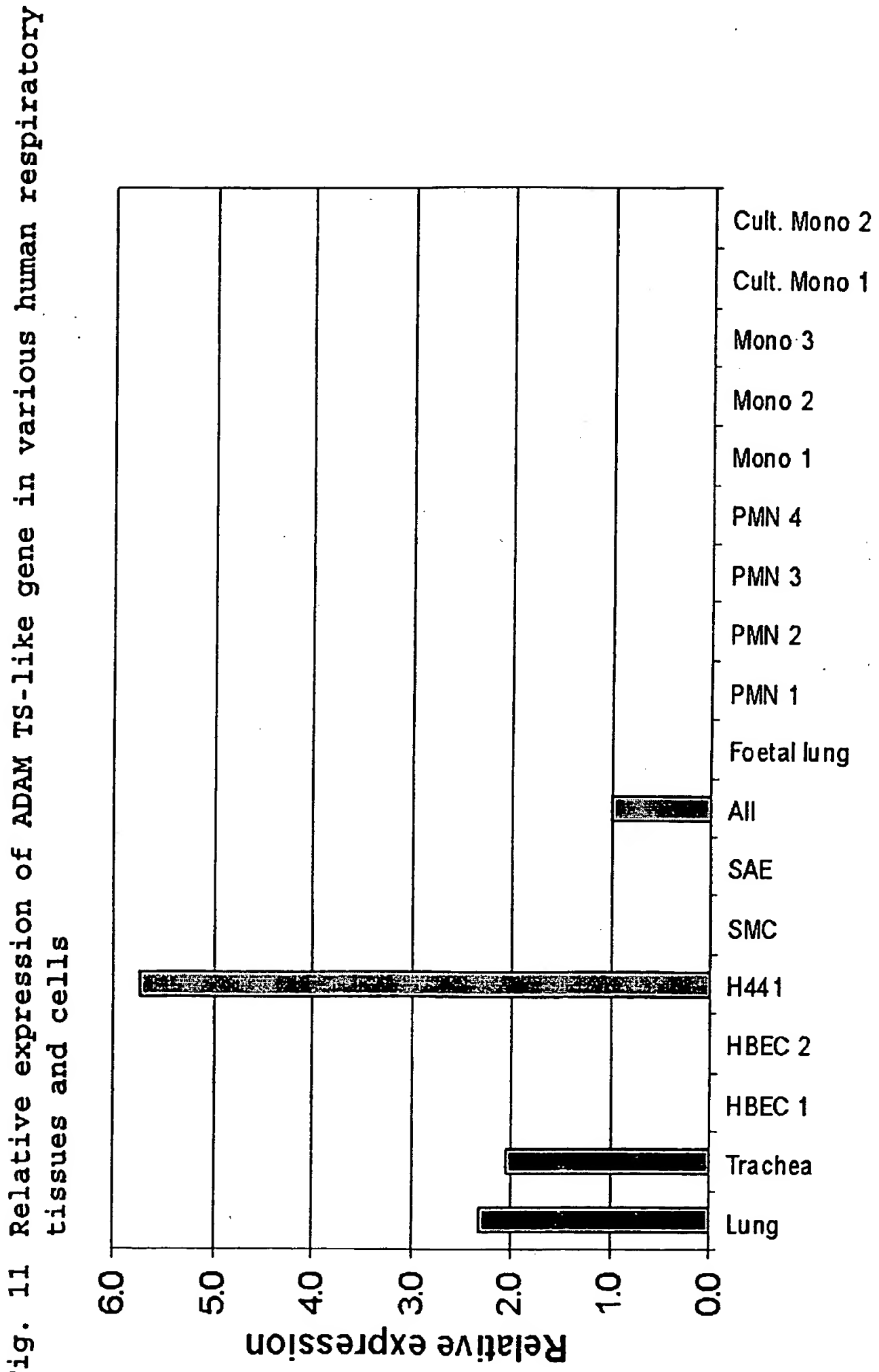


Fig. 11 Relative expression of ADAM TS-like gene in various human respiratory tissues and cells

SEQUENCE LISTING

<110> Bayer AG

<120> REGULATION OF HUMAN ADAM-TS-LIKE ZINC METALLOPROTEASE

<130> LIO274 Foreign Countries

<150> US 60/262,034

<151> 2001-01-18

<160> 7

<170> PatentIn version 3.1

<210> 1

<211> 1668

<212> DNA

<213> Homo sapiens

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<222> (1)..(1668)

<223>

<400> 1

```
aat gaa gaa ctg aac gtg gag acc ttg gtg gtg gtc gac aaa aag atg       48
Asn Glu Glu Leu Asn Val Glu Thr Leu Val Val Val Asp Lys Lys Met
1               5                  10                  15

atg caa aac cat ggc cat gaa aat atc acc acc tac gtg ctc acg ata       96
Met Gln Asn His Gly His Glu Asn Ile Thr Thr Tyr Val Leu Thr Ile
            20                  25                  30
```

```
ctc aac atg gta tct gct tta ttc aaa gat gga aca ata gga gga aac      144
Leu Asn Met Val Ser Ala Leu Phe Lys Asp Gly Thr Ile Gly Gly Asn
        35                  40                  45

atc aac att gca att gta ggt ctg att ctt cta gaa gat gaa cag cca      192
Ile Asn Ile Ala Ile Val Gly Leu Ile Leu Leu Glu Asp Glu Gln Pro
    50                  55                  60

gga ctg gtg ata agt cac cac gca gac cac acc tta agt agc ttc tgc      240
Gly Leu Val Ile Ser His His Ala Asp His Thr Leu Ser Ser Phe Cys
65                  70                  75                  80

cag tgg cag tct gga ttg atg ggg aaa gat ggg act cgt cat gac cac      288
Gln Trp Gln Ser Gly Leu Met Gly Lys Asp Gly Thr Arg His Asp His
                85                  90                  95

gcc atc tta ctg act ggt ctg gat ata tgt tcc tgg aag aat gag ccc      336
Ala Ile Leu Leu Thr Gly Leu Asp Ile Cys Ser Trp Lys Asn Glu Pro
            100                 105                 110

tgt gac act ttg gga ttt gca ccc ata agt gga atg tgt agt aaa tat      384
Cys Asp Thr Leu Gly Phe Ala Pro Ile Ser Gly Met Cys Ser Lys Tyr
        115                 120                 125

cgc agc tgc acg att aat gaa gat aca ggt ctt gga ctg gcc ttc acc      432
Arg Ser Cys Thr Ile Asn Glu Asp Thr Gly Leu Gly Leu Ala Phe Thr
    130                 135                 140

att gcc cat gag tct gga cac aac ttt ggc atg att cat gat gga gaa      480
Ile Ala His Glu Ser Gly His Asn Phe Gly Met Ile His Asp Gly Glu
145                 150                 155                 160

ggg aac atg tgc aaa aag tcc gag ggc aac atc atg tcc cct aca ttg      528
Gly Asn Met Cys Lys Lys Ser Glu Gly Asn Ile Met Ser Pro Thr Leu
                165                 170                 175

gca gga cgc aat gga gtc ttc tcc tgg tca ccc tgc agc cgc cag tat      576
Ala Gly Arg Asn Gly Val Phe Ser Trp Ser Pro Cys Ser Arg Gln Tyr
            180                 185                 190

cta cac aaa ttt cta agc acc gct caa gct atc tgc ctt gct gat cag      624
Leu His Lys Phe Leu Ser Thr Ala Gln Ala Ile Cys Leu Ala Asp Gln
        195                 200                 205

cca aag cct gtg aag gaa tac aag tat cct gag aaa ttg cca gga gaa      672
Pro Lys Pro Val Lys Glu Tyr Lys Tyr Pro Glu Lys Leu Pro Gly Glu
    210                 215                 220

tta tat gat gca aac aca cag tgc aag tgg cag ttc gga gag aaa gcc      720
Leu Tyr Asp Ala Asn Thr Gln Cys Lys Trp Gln Phe Gly Glu Lys Ala
225                 230                 235                 240

aag ctc tgc atg ctg gac ttt aaa aag gca acc ctg tgg tgc cat cgt      768
Lys Leu Cys Met Leu Asp Phe Lys Lys Ala Thr Leu Trp Cys His Arg
                245                 250                 255

att gga agg aaa tgt gag act aaa ttt atg cca gca gca gaa ggc aca      816
Ile Gly Arg Lys Cys Glu Thr Lys Phe Met Pro Ala Ala Glu Gly Thr
            260                 265                 270
```

```
att tgt ggg cat gac atg tgg tgc cgg gga gga cag tgt gtg aaa tat      864
Ile Cys Gly His Asp Met Trp Cys Arg Gly Gly Gln Cys Val Lys Tyr
        275                 280                 285

ggt gat gaa ggc ccc aag ccc acc cat ggc cac tgg tcg gac tgg tct      912
Gly Asp Glu Gly Pro Lys Pro Thr His Gly His Trp Ser Asp Trp Ser
    290                 295                 300

tct tgg tcc cca tgc tcc agg acc tgc gga ggg gga gta tct cat agg      960
Ser Trp Ser Pro Cys Ser Arg Thr Cys Gly Gly Gly Val Ser His Arg
305                 310                 315                 320

agt cgc ctc tgc acc aac ccc aag cca tcg cat gga ggg aag ttc tgt     1008
Ser Arg Leu Cys Thr Asn Pro Lys Pro Ser His Gly Gly Lys Phe Cys
                325                 330                 335

gag ggc tcc act cgc act ctg aag ctc tgc aac agt cag aaa tgt ccc     1056
Glu Gly Ser Thr Arg Thr Leu Lys Leu Cys Asn Ser Gln Lys Cys Pro
            340                 345                 350

cgg gac agt gtt gac ttc cgt gct gct cag tgt gcc gag cac aac agc     1104
Arg Asp Ser Val Asp Phe Arg Ala Ala Gln Cys Ala Glu His Asn Ser
        355                 360                 365

aga cga ttc aga ggg cgg cac tac aag tgg aag cct tac act caa gta     1152
Arg Arg Phe Arg Gly Arg His Tyr Lys Trp Lys Pro Tyr Thr Gln Val
    370                 375                 380

gaa gat cag gac tta tgc aaa ctc tac tgt atc gca gaa gga ttt gat     1200
Glu Asp Gln Asp Leu Cys Lys Leu Tyr Cys Ile Ala Glu Gly Phe Asp
385                 390                 395                 400

ttc ttc ttt tct ttg tca aat aaa gtc aaa gat ggg act cca tgc tcg     1248
Phe Phe Phe Ser Leu Ser Asn Lys Val Lys Asp Gly Thr Pro Cys Ser
                405                 410                 415

gag gat agc cgt aat gtt tgt ata gat ggg ata tgt gag aga gtt gga     1296
Glu Asp Ser Arg Asn Val Cys Ile Asp Gly Ile Cys Glu Arg Val Gly
            420                 425                 430

tgt gac aat gtc ctt gga tct gat gct gtt gaa gac gtc tgt ggg gtg     1344
Cys Asp Asn Val Leu Gly Ser Asp Ala Val Glu Asp Val Cys Gly Val
        435                 440                 445

tgt aac ggg aat aac tca gcc tgc acg att cac agg ggt ctc tac acc     1392
Cys Asn Gly Asn Asn Ser Ala Cys Thr Ile His Arg Gly Leu Tyr Thr
    450                 455                 460

aag cac cac cac acc aac cag tat tat cac atg gtc acc att cct tct     1440
Lys His His His Thr Asn Gln Tyr Tyr His Met Val Thr Ile Pro Ser
465                 470                 475                 480

gga gcc cgg agt atc cgc atc tat gaa atg aac gtc tct acc tcc tac     1488
Gly Ala Arg Ser Ile Arg Ile Tyr Glu Met Asn Val Ser Thr Ser Tyr
                485                 490                 495
```

```
att tct gtg cgc aat gcc ctc aga agg tac tac ctg aat ggg cac tgg      1536
Ile Ser Val Arg Asn Ala Leu Arg Arg Tyr Tyr Leu Asn Gly His Trp
          500                 505                 510

acc gtg gac tgg ccc ggc cgg tac aaa ttt tcg ggc act act ttc gac      1584
Thr Val Asp Trp Pro Gly Arg Tyr Lys Phe Ser Gly Thr Thr Phe Asp
        515                 520                 525

tac aga cgg tcc tat aat gag ccc gag aac tta atc gct act gga cca      1632
Tyr Arg Arg Ser Tyr Asn Glu Pro Glu Asn Leu Ile Ala Thr Gly Pro
    530                 535                 540

acc aac gag aca ctg att gtg gag gta aag tcc agc                      1668
Thr Asn Glu Thr Leu Ile Val Glu Val Lys Ser Ser
545                 550                 555
```

<210> 2

<211> 556

<212> PRT

<213> Homo sapiens

<400> 2

```
Asn Glu Glu Leu Asn Val Glu Thr Leu Val Val Val Asp Lys Lys Met
1               5                   10                  15

Met Gln Asn His Gly His Glu Asn Ile Thr Thr Tyr Val Leu Thr Ile
            20                  25                  30

Leu Asn Met Val Ser Ala Leu Phe Lys Asp Gly Thr Ile Gly Gly Asn
        35                  40                  45

Ile Asn Ile Ala Ile Val Gly Leu Ile Leu Leu Glu Asp Glu Gln Pro
    50                  55                  60

Gly Leu Val Ile Ser His His Ala Asp His Thr Leu Ser Ser Phe Cys
65                  70                  75                  80

Gln Trp Gln Ser Gly Leu Met Gly Lys Asp Gly Thr Arg His Asp His
                85                  90                  95

Ala Ile Leu Leu Thr Gly Leu Asp Ile Cys Ser Trp Lys Asn Glu Pro
            100                 105                 110

Cys Asp Thr Leu Gly Phe Ala Pro Ile Ser Gly Met Cys Ser Lys Tyr
        115                 120                 125
```

```
Arg Ser Cys Thr Ile Asn Glu Asp Thr Gly Leu Gly Leu Ala Phe Thr
    130                 135                 140

Ile Ala His Glu Ser Gly His Asn Phe Gly Met Ile His Asp Gly Glu
145                 150                 155                 160

Gly Asn Met Cys Lys Lys Ser Glu Gly Asn Ile Met Ser Pro Thr Leu
                165                 170                 175

Ala Gly Arg Asn Gly Val Phe Ser Trp Ser Pro Cys Ser Arg Gln Tyr
            180                 185                 190

Leu His Lys Phe Leu Ser Thr Ala Gln Ala Ile Cys Leu Ala Asp Gln
        195                 200                 205

Pro Lys Pro Val Lys Glu Tyr Lys Tyr Pro Glu Lys Leu Pro Gly Glu
    210                 215                 220

Leu Tyr Asp Ala Asn Thr Gln Cys Lys Trp Gln Phe Gly Glu Lys Ala
225                 230                 235                 240

Lys Leu Cys Met Leu Asp Phe Lys Lys Ala Thr Leu Trp Cys His Arg
                245                 250                 255

Ile Gly Arg Lys Cys Glu Thr Lys Phe Met Pro Ala Ala Glu Gly Thr
            260                 265                 270

Ile Cys Gly His Asp Met Trp Cys Arg Gly Gly Gln Cys Val Lys Tyr
        275                 280                 285

Gly Asp Glu Gly Pro Lys Pro Thr His Gly His Trp Ser Asp Trp Ser
    290                 295                 300

Ser Trp Ser Pro Cys Ser Arg Thr Cys Gly Gly Gly Val Ser His Arg
305                 310                 315                 320

Ser Arg Leu Cys Thr Asn Pro Lys Pro Ser His Gly Gly Lys Phe Cys
                325                 330                 335

Glu Gly Ser Thr Arg Thr Leu Lys Leu Cys Asn Ser Gln Lys Cys Pro
            340                 345                 350
```

```
Arg Asp Ser Val Asp Phe Arg Ala Ala Gln Cys Ala Glu His Asn Ser
        355                 360                 365

Arg Arg Phe Arg Gly Arg His Tyr Lys Trp Lys Pro Tyr Thr Gln Val
    370                 375                 380

Glu Asp Gln Asp Leu Cys Lys Leu Tyr Cys Ile Ala Glu Gly Phe Asp
385                 390                 395                 400

Phe Phe Phe Ser Leu Ser Asn Lys Val Lys Asp Gly Thr Pro Cys Ser
                405                 410                 415

Glu Asp Ser Arg Asn Val Cys Ile Asp Gly Ile Cys Glu Arg Val Gly
            420                 425                 430

Cys Asp Asn Val Leu Gly Ser Asp Ala Val Glu Asp Val Cys Gly Val
        435                 440                 445

Cys Asn Gly Asn Asn Ser Ala Cys Thr Ile His Arg Gly Leu Tyr Thr
    450                 455                 460

Lys His His His Thr Asn Gln Tyr Tyr His Met Val Thr Ile Pro Ser
465                 470                 475                 480

Gly Ala Arg Ser Ile Arg Ile Tyr Glu Met Asn Val Ser Thr Ser Tyr
                485                 490                 495

Ile Ser Val Arg Asn Ala Leu Arg Arg Tyr Tyr Leu Asn Gly His Trp
            500                 505                 510

Thr Val Asp Trp Pro Gly Arg Tyr Lys Phe Ser Gly Thr Thr Phe Asp
        515                 520                 525

Tyr Arg Arg Ser Tyr Asn Glu Pro Glu Asn Leu Ile Ala Thr Gly Pro
    530                 535                 540

Thr Asn Glu Thr Leu Ile Val Glu Val Lys Ser Ser
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<212> PRT

<213> Mus musculus

<400> 3

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His Met Leu Leu Leu Leu Leu Ala Ser Ile Thr Met Leu Leu Cys Ala
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Arg Gly Ala His Gly Arg Pro Thr Glu Glu Asp Glu Glu Leu Val Leu
35 40 45

Pro Ser Leu Glu Arg Ala Pro Gly His Asp Ser Thr Thr Thr Arg Leu
50 55 60

Arg Leu Asp Ala Phe Gly Gln Gln Leu His Leu Lys Leu Gln Pro Asp
65 70 75 80

Ser Gly Phe Leu Ala Pro Gly Phe Thr Leu Gln Thr Val Gly Arg Ser
85 90 95

Pro Gly Ser Glu Ala Gln His Leu Asp Pro Thr Gly Asp Leu Ala His
100 105 110

Cys Phe Tyr Ser Gly Thr Val Asn Gly Asp Pro Gly Ser Ala Ala Ala
115 120 125

Leu Ser Leu Cys Glu Gly Val Arg Gly Ala Phe Tyr Leu Gln Gly Glu
130 135 140

Glu Phe Phe Ile Gln Pro Ala Pro Gly Val Ala Thr Glu Arg Leu Ala
145 150 155 160

Pro Ala Val Pro Glu Glu Glu Ser Ser Ala Arg Pro Gln Phe His Ile
165 170 175

Leu Arg Arg Arg Arg Arg Gly Ser Gly Gly Ala Lys Cys Gly Val Met
180 185 190

```
Asp Asp Glu Thr Leu Pro Thr Ser Asp Ser Arg Pro Glu Ser Gln Asn
        195                 200                 205

Thr Arg Asn Gln Trp Pro Val Arg Asp Pro Thr Pro Gln Asp Ala Gly
    210                 215                 220

Lys Pro Ser Gly Pro Gly Ser Ile Arg Lys Lys Arg Phe Val Ser Ser
225                 230                 235                 240

Pro Arg Tyr Val Glu Thr Met Leu Val Ala Asp Gln Ser Met Ala Asp
                245                 250                 255

Phe His Gly Ser Gly Leu Lys His Tyr Leu Leu Thr Leu Phe Ser Val
            260                 265                 270

Ala Ala Arg Phe Tyr Lys His Pro Ser Ile Arg Asn Ser Ile Ser Leu
        275                 280                 285

Val Val Val Lys Ile Leu Val Ile Tyr Glu Glu Gln Lys Gly Pro Glu
    290                 295                 300

Val Thr Ser Asn Ala Ala Leu Thr Leu Arg Asn Phe Cys Asn Trp Gln
305                 310                 315                 320

Lys Gln His Asn Ser Pro Ser Asp Arg Asp Pro Glu His Tyr Asp Thr
                325                 330                 335

Ala Ile Leu Phe Thr Arg Gln Asp Leu Cys Gly Ser His Thr Cys Asp
            340                 345                 350

Thr Leu Gly Met Ala Asp Val Gly Thr Val Cys Asp Pro Ser Arg Ser
        355                 360                 365

Cys Ser Val Ile Glu Asp Asp Gly Leu Gln Ala Ala Phe Thr Thr Ala
    370                 375                 380

His Glu Leu Gly His Val Phe Asn Met Pro His Asp Asp Ala Lys His
385                 390                 395                 400

Cys Ala Ser Leu Asn Gly Val Thr Gly Asp Ser His Leu Met Ala Ser
                405                 410                 415

Met Leu Ser Ser Leu Asp His Ser Gln Pro Trp Ser Pro Cys Ser Ala
            420                 425                 430
```

```
Tyr Met Val Thr Ser Phe Leu Asp Asn Gly His Gly Glu Cys Leu Met
        435                 440                 445

Asp Lys Pro Gln Asn Pro Ile Lys Leu Pro Ser Asp Leu Pro Gly Thr
    450                 455                 460

Leu Tyr Asp Ala Asn Arg Gln Cys Gln Phe Thr Phe Gly Glu Glu Ser
465                 470                 475                 480

Lys His Cys Pro Asp Ala Ala Ser Thr Cys Thr Thr Leu Trp Cys Thr
                485                 490                 495

Gly Thr Ser Gly Gly Leu Leu Val Cys Gln Thr Lys His Phe Pro Trp
            500                 505                 510

Ala Asp Gly Thr Ser Cys Gly Glu Gly Lys Trp Cys Val Ser Gly Lys
        515                 520                 525

Cys Val Asn Lys Thr Asp Met Lys His Phe Ala Thr Pro Val His Gly
    530                 535                 540

Ser Trp Gly Pro Trp Gly Pro Trp Gly Asp Cys Ser Arg Thr Cys Gly
545                 550                 555                 560

Gly Gly Val Gln Tyr Thr Met Arg Glu Cys Asp Asn Pro Val Pro Lys
                565                 570                 575

Asn Gly Gly Lys Tyr Cys Glu Gly Lys Arg Val Arg Tyr Arg Ser Cys
            580                 585                 590

Asn Ile Glu Asp Cys Pro Asp Asn Asn Gly Lys Thr Phe Arg Glu Glu
        595                 600                 605

Gln Cys Glu Ala His Asn Glu Phe Ser Lys Ala Ser Phe Gly Asn Glu
    610                 615                 620

Pro Thr Val Glu Trp Thr Pro Lys Tyr Ala Gly Val Ser Pro Lys Asp
625                 630                 635                 640

Arg Cys Lys Leu Thr Cys Glu Ala Lys Gly Ile Gly Tyr Phe Phe Val
                645                 650                 655
```

```
Leu Gln Pro Lys Val Val Asp Gly Thr Pro Cys Ser Pro Asp Ser Thr
            660                 665                 670

Ser Val Cys Val Gln Gly Gln Cys Val Lys Ala Gly Cys Asp Arg Ile
        675                 680                 685

Ile Asp Ser Lys Lys Lys Phe Asp Lys Cys Gly Val Cys Gly Gly Asn
    690                 695                 700

Gly Ser Thr Cys Lys Lys Met Ser Gly Ile Val Thr Ser Thr Arg Pro
705                 710                 715                 720

Gly Tyr His Asp Ile Val Thr Ile Pro Ala Gly Ala Thr Asn Ile Glu
                725                 730                 735

Val Lys His Arg Asn Gln Arg Gly Ser Arg Asn Asn Gly Ser Phe Leu
            740                 745                 750

Ala Ile Arg Ala Ala Asp Gly Thr Tyr Ile Leu Asn Gly Asn Phe Thr
        755                 760                 765

Leu Ser Thr Leu Glu Gln Asp Leu Thr Tyr Lys Gly Thr Val Leu Arg
    770                 775                 780

Tyr Ser Gly Ser Ser Ala Ala Leu Glu Arg Ile Arg Ser Phe Ser Pro
785                 790                 795                 800

Leu Lys Glu Pro Leu Thr Ile Gln Val Leu Met Val Gly His Ala Leu
                805                 810                 815

Arg Pro Lys Ile Lys Phe Thr Tyr Phe Met Lys Lys Lys Thr Glu Ser
            820                 825                 830

Phe Asn Ala Ile Pro Thr Phe Ser Glu Trp Val Ile Glu Glu Trp Gly
        835                 840                 845

Glu Cys Ser Lys Thr Cys Gly Ser Gly Trp Gln Arg Arg Val Val Gln
    850                 855                 860

Cys Arg Asp Ile Asn Gly His Pro Ala Ser Glu Cys Ala Lys Glu Val
865                 870                 875                 880

Lys Pro Ala Ser Thr Arg Pro Cys Ala Asp Leu Pro Cys Pro His Trp
                885                 890                 895
```

Gln Val Gly Asp Trp Ser Pro Cys Ser Lys Thr Cys Gly Lys Gly Tyr
900 905 910

Lys Lys Arg Thr Leu Lys Cys Val Ser His Asp Gly Gly Val Leu Ser
915 920 925

Asn Glu Ser Cys Asp Pro Leu Lys Lys Pro Lys His Tyr Ile Asp Phe
930 935 940

Cys Thr Leu Thr Gln Cys Ser
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<212> DNA

<213> Homo sapiens

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<211> 20

<212> DNA

<213> Homo sapiens

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<400> 5
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<210> 6

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<213> Homo sapiens

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<223> Reverse primer

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<223> Reverse primer for expression profiling

<400> 6
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<210> 7

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<212> DNA

<213> Homo sapiens

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<221> misc_feature

<223> Probe for expression profiling

<400> 7
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